Bioengineering Requirements for the Intensive Culture of California Halibut (*Paralichthys californicus*).

By

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Culture of California Halibut (*Paralichthys californicus*).

Abstract

Bioengineering parameters were determined for California halibut in a marine recirculating system under farm-like conditions. California halibut studied ranged in size from 1 to 400 g. Bio-engineering parameters studied were relative swimming velocity; stocking density; oxygen consumption rates; ammonia and urea excretion rates; and feces and uneaten feed settling velocities.

California halibut were reared at a relative swimming velocity between 0.5 and 1.5 body length per second (bl/s). It was determined that a velocity up to 1.0 bl/s was adequate to achieve maximum fish growth. Stocking densities between 100 and 300 percent of coverage area (PCA) were tested. It was found that maximum fish growth was maintained at densities up to 200% PCA. Daily average oxygen consumption rates ranged from 0.31 to 1.40 g O_2 / g feed, ammonia as N (TAN) excretion rate values ranged from 4.3 to 8.5 mg TAN / g feed, and urea as N (urea-N) excretion rate values ranged from 0.8 to 1.8 mg urea-N / g feed.

Settling velocity experiments were performed in a top-loading settling column with solids that were already settled within the raceways. Settling velocities were found to vary among feeds used regardless of fish size. Average settling velocities were 1.7 cm/s for Nippai^{TM} 600, 2.2 cm/s for BioKyowa^{TM} 1000, 4.4 cm/s for BioKyowa^{TM} 2000, 1.7 cm/s for EWOS^{TM} 2, and 2.0 cm/s for EWOS 3. At these settling velocities, between 90 and 98% of suspended solids by mass will settle out in a quiescent zone of a culture tank having an overflow rate less than 0.31 cm/s.

The performance of a submerged moving bed biofilter (SMBB) was also studied under farm-like conditions. The biofilter had a hydraulic loading rate of 0.13 m^3/m^2 d, a maximum volumetric nitrification rate of 61 g/m^3 d and a maximum surface nitrification rate of 0.10 g/m^2 d. Finally, a Recirculating Pilot System was designed to produce 620 fish at 1000 g mean mass with the use of the bio-engineering data and the SMBB performance gathered in this research.

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CHAPTER I

INTRODUCTION

The California halibut (*Paralichthys californicus*) is one of the most economically important flatfish species of Southern California in the USA (Gadomski et al., 1990) and Baja California in Mexico (Hammann and Ramirez, 1990). In the early 1980's, concerns over the effects of the overfishing of natural stocks and the uncertainties and risks associated with the fishery activity (Wallace, 1990) led to the development of hatcheries for the production of California halibut for re-stocking. The California Halibut Hatchery Program (Redondo Beach, California) and, lately, the Hubbs - Sea World Research Institute (Carlsbad, California) have been the only two hatcheries for California halibut (Hobbs et al., 1990).

With the existence of the California Halibut Hatchery at Redondo Beach, techniques were developed for broodstock conditioning, which resulted in producing a reliable supply of eggs and larvae (Caddel et al., 1990). Basic information has been gathered concerning the development of the California halibut's early life history (Gadomski and Caddel, 1991) and juvenile temperature preferences (Innis, 1980) with the ultimate purpose of enhancing ocean populations through juvenile release (Jim Rounds, pers. comm.). The Hubbs - Sea World Research Institute (Carlsbad, California) focused their research efforts towards the development of a technology for culturing juvenile California halibut

in shallow raceways, and first attempts for weaning were made, with no success for juveniles weighing up to 100 mg (Oiestad, 1999; Jirsa and Drawbridge, 2000). In the early 2000s, at the University of California at Davis in association with the California Halibut Hatchery Program at Redondo Beach, and supported by the California Sea Grant College Program, a research project was put together to elucidate biological variables and bioengineering parameters for California halibut which are essential for the design of commercial recirculation aquaculture system (RAS) facilities and the development of growout techniques.

Although California halibut is a popular commercial and sport species in California, no work on experimental or pilot aquaculture operations has been published to stimulate interest in the commercial production of this flatfish. Information on the factors that affect the growth and survival of California halibut is needed for its successful aquaculture. Those factors include temperature (Kikuchi and Kurokura, 1995), food quality and availability (Kikuchi et al., 1992; Kikuchi et al., 1993), photoperiod (Imsland et al., 1995), container size (Cripps and Poxton, 1992), fish density (Jeon et al., 1993), and water quality (Kikuchi et al., 1991). The successful commercialization of hirame (*Paralichthys olivaceus*) in Asia and turbot (*Scophthalmus maximus*) culture in Europe (Person-Le Ruyet et al., 1981; Joseph, 1990; Kikuchi, 1995) can provide a model for research focused on the aquaculture of California halibut. This model has also being followed by the Chilean aquaculture industry to develop the culture of their local flatfish to diversify their aquacultural industry (Alvial and Manriquez, 1999).

There are a few works previously published regarding the effect of environmental variables on growth performance in California halibut juveniles (Innis, 1980; Madon, 2002). According to Madon (2002), growth rates of California halibut between 11.8 and 17.2 cm TL, did not vary significantly under a combination of salinity (17, and 34 g/L) and temperature (20, 25, and 28 °C). Madon (2002) reported that early juveniles can tolerate a greater range of salinities and temperatures than do older juveniles, which agrees with previous work done by Innis (1980). Several studies suggest that the upper temperature for all stages is 28 °C (Innis 1980; Gadomski and Caddel, 1991; Madon, 2002).

The most important biological processes that affect water quality in fish tanks include respiration (oxygen consumption and carbon dioxide production), nitrogen excretion and fecal production (Huguenin and Colt, 1989; Timmons et al., 2001). These processes generate fundamental bio-engineering design variables for fish culture systems and for aquaculture waste treatment operations (Muir, 1982). Bioengineering information on fish oxygen consumption and metabolite/fecal excretion rates is used to design and manage fish culture systems (Kikuchi, 1995). Conversely, data on water quality changes in a system might be used to obtain information on the bioengineering parameters.

Landbased farms require high investments and are expensive to operate (Kjarttansson, 1993). Given the high capital costs, there is a tendency to use high

stocking densities while minimizing the investment for treatment facilities and utilizing them at their maximum loading capacity. Therefore, it is of great economic importance to find the maximum densities at which the fish can be produced. Stocking density for flatfish might be expressed either as a percentage of coverage area (PCA = total fish ventral area to total tank bottom area ratio) or as biomass per unit bottom area. It has been reported that flatfish can be grown in densities up to 300% of coverage area and over 120 kg/m^2 (Jones, 1981; Person Le-Ruyet et al, 1983; Liewes, 1984; Martinez-Tapia and Fernandez-Pato, 1991; Adoff et al., 1993; Honda, 1998; Jeon et al., 1993; King et al, 1998; Mallekh et al., 1998; Silva and Velez, 1998; Kikuchi, 2000).

The water flow requirement is biologically important in land-based systems since it affects many of the limiting factors for fish production (Brown et al., 1984; Fivelstad, 1988; Hallaraker et al., 1995; Imsland et al., 1995; Thomas and Piedrahita, 1997, 1998; Fivelstad et al., 1999). In the design of a fish culture facility, oxygen consumption by fish must be known to estimate either water requirements or aeration/oxygenation devices required to provide the oxygen needed by the fish (Lawson, 1995; Timmons et al., 2001).

Water flow requirements to meet fish oxygen demands and remove metabolic waste products may induce high water velocities in a culture tank, which can affect fish growth and efficiency of feed utilization. A few reports on flatfish, under farm-like conditions, recommend that relative swimming velocities not be

over 1.0 bl/s (body length per second) (Ogata and Oku, 2000; Bengston and Alves, 2002).

Treatment of wastes must be an integral part of water reuse systems. Suspended particles in recirculating aquaculture systems are produced within the system (Smith et al., 1980; Beveridge et al., 1991; Bergheim and Brinker, 2003; Patterson and Watts, 2003). Hence an important component of waste treatment is removal of solids, which are excess feed and fecal material. Sedimentation is a common process used for this purpose. Characterization of the settling velocity of particles is essential for the design of effective solids removal systems.

When oxygen requirements of fish can be met by mechanical devices or oxygenation systems, the next major concern in the design of systems to reuse water is the excretion of the metabolic byproducts by the fish (Colt and Armstrong, 1981). To design an effective marine recirculating system to grow fish, it is important to examine their nitrogen excretion rate. The nitrogen byproducts of main concern are ammonia (Alderson, 1979; Wajsbrot et al., 1993; Person-Le Ruyet et al., 1995; Person-Le Ruyet et al., 1997) and urea (Kikuchi et al., 1990; Kikuchi, 1995; Dosdat el at., 1996; Verbeeten et al., 1999).

Developments in the commercial culture of California halibut have been constrained by a lack of the bioengineering information necessary for the design of fish culture systems. The design of a commercially viable system involves

considerations beyond pure engineering criteria for integrating the elements into a working physical system (Huguenin and Colt, 1989). Effective system design can be realized only if the bioengineering criteria for design are well understood. The methods used in this research are applicable to the determination of bioengineering parameters that will assist in aquacultural facility design and management for the production of juvenile California halibut. The main goal of this research will be to further our understanding of the bioengineering requirements of California halibut juveniles and to apply that information to the design of rearing facilities for their intensive culture.

CHAPTER II

OBJECTIVES OF THE STUDY

1) To evaluate the effects of water flow velocity on the growth rate of California halibut juveniles (Chapter IV)
2) To evaluate the effects of fish stocking density on the growth rate of California halibut juveniles (Chapter V)
3) To determine the oxygen consumption rates of California halibut juveniles (Chapter VI)
4) To determine ammonia and urea excretion rates of California halibut juveniles (Chapter VII)
5) To characterize the settling velocity distribution of particulate waste products settled within the culture vessels of California halibut juveniles (Chapter VIII)
6) To determine the nitrification performance of a submerged moving bed biofilter (Chapter IX)
7) To design a pilot culture facility to growout California halibut juveniles (Chapter X)

CHAPTER III

LITERATURE REVIEW

3.1.- INTRODUCTION

Recirculating aquaculture systems (RAS) are a type of intensive fish culture technology in which a high percentage of the water is reused after treatment. A RAS was defined by Losordo (1991) as a production unit that replace less than 10% of the total system volume on a daily basis. The biological parameters of primary concern for RAS engineering design are oxygen, ammonia, suspended solids and carbon dioxide (Heinen et al., 1996; Timmons et al., 2001). These parameters are known as bio-engineering parameters by designer and development aquaculture engineers.

With a worldwide growing interest in tank based production systems, design approaches based on water quality parameters and fish metabolism were put in practice in the 1970s to design reliable inland facilities (Willoughby, 1968; Buss and Miller, 1971; Speece, 1973; Westers and Pratt, 1977). Water flow requirement in a land based system was recognized as the limiting factor for fish production. Willoughby (1968) regarded oxygen as the first limiting factor which determined that requirement. Willoughby et al. (1972) found that ammonia production was proportional to the amount of feed given. Speece (1973)

determined that ammonia was a limiting factor in water reuse aquaculture systems. Liao and Mayo (1974) reported that high levels of suspended solids interfere with a filter's nitrification performance and recommended its immediate removal from the culture water since about 70% of ammonia present was associated with organic solids mineralization. Westers and Pratt (1977) considered both oxygen consumption and ammonia excretion as limiting factors, depending of the environmental conditions. Water quality criteria became the basis for designing recirculating aquaculture systems (RAS).

The four critical processes for reconditioning of water in recirculating fish culture systems are: (1) gas exchange to ensure sufficient oxygen supply for fish and biological filtration and to strip carbon dioxide; (2) solids removal to remove fecal wastes, uneaten feed, and excess bacterial biomass; (3) biofiltration, primarily nitrification to convert ammonia to nitrate; and (4) ion balance, primarily to maintain pH and alkalinity (Lucchetty and Gray, 1988; Huguening and Colt, 1989; Losordo, 1991; Rosenthal and Black, 1993; Timmons and Losordo, 1994; Malone and DeLosReyes, 1997; Summerfelt and Wade, 1997). Additional treatment components including denitrification, ozonation, disinfection, and foam fractionation may be provided to meet specific production needs (Timmons et al., 2001). The present review will show the effect of fish rearing on water quality changes, and therefore the importance in determining bioengineering parameters, and how these can be included in mass balances analysis to design a reliable RAS for the intensive culture of fish.

3.2.- WATER QUALITY CHANGES DUE TO FISH REARING

In the planning of a fish culture setting, the degradation of water quality due to high fish density is a central issue in the design and operation of facilities (Pennell and McClean, 1996). High stocking densities will result in significant changes in the concentrations of dissolved oxygen, ammonia, carbon dioxide, and suspended solids as water passes through the facility (Timmons and Youngs, 1991; Timmons et al., 1998).

3.2.1.- Oxygen consumption

Oxygen consumption in fish generally increases with increasing water temperature (Jobling, 1994), but exceptions to this generalization have been reported (Forsberg, 1994). The oxygen consumption rates for turbot (*Scophthalmus maximus*) (400 to 600 g mass) at different temperatures was found to increase from 6 to 18 °C, but was almost constant between 18 and 22 °C (Mallekh and Lagardere, 2002). Mallekh and Lagardere (2002) reported 5.64 g O_2 kg^{-1} d^{-1} as the maximum oxygen consumption rate when fed turbot were forced to swim at temperatures of 18 to 22 ° C.

In fish with exposure to increased water velocities the physiological response to exercise is often reported to induce an increase in oxygen consumption (Brett, 1964; Smith et al., 1971; Christiansen and Jobling, 1990; Christiansen et al., 1991). This has been observed in rainbow trout (*Oncorhynchus mykiss*) (Alsop

and Wood, 1997), Nile tilapia (*Oreochromis niloticus*) (Alsop et al., 1999), common flounder (*Platichthys flesus*), common dab (*Limanda limanda*), and lemon sole (*Microstomus kitt*) (Duthie, 1982).

Research on fish aggregation has shown that oxygen consumption of fish varies with the number of fish in the group (Kanda and Itazawa, 1981; Umezawa et al., 1983). Parker (1973) attributed this phenomenon to an interaction of a calming effect and a possible hydrodynamic effect. Honda (1988) reported that oxygen consumption in hirame (*Paralichthys olivaceus*) held singly was 11 to 17% greater than that of fish held in groups. Hence studies of oxygen consumption with single fish may overestimate the real needs of oxygen supplies and furthermore increase the investment cost of a commercial farm (Brown et al., 1984; Forsberg, 1994; Thomas and Piedrahita, 1997).

In aquaculture systems diurnal variations in oxygen consumption have been related to feeding activity for sockeye salmon (*Oncorhynchus nerka*) (Brett and Zala, 1975), Atlantic salmon (*Salmo salar*) (Bergheim et al., 1991), rainbow trout (Wagner et al., 1995), white sturgeon (*Acipenser transmontanus*) (Thomas and Piedrahita, 1997), and sea bass (*Dicentrarchus labrax*) (Tudor, 1999). Photoperiod length also affected the oxygen consumption in juvenile turbot (Waller, 1992; Imsland et al,. 1995). Since oxygen consumption varies during the day it might lead to oxygen concentration changes. Oxygen below critical concentrations may cause severe stress in fish, leading to appetite reduction and

growth depression (Carlson et al., 1980). Significantly lower growth rates in juvenile winter flounder (*Pleuronectes americanus*) were reported for a dissolved oxygen environment cycling from 2.5 to 6.4 mg/L at 18.7 °C (Bejda et al., 1992). For turbot it was found that oxygen consumption was constant over the range of 60-100% of saturation for temperatures within 7-16 °C (Brown et al, 1984). For juvenile common flounder (*Paralichthys flesus*), an oxygen concentration below 30% saturation caused a decrease in predation efficiency (Tallqvist et al., 1999). For sole (*Solea solea*) a decrease in activity was recorded at an oxygen saturation of 40% (Van der Thillart et al., 1994). Decreased growth for plaice (*Pleuronectes platessa*) and common dab was recorded at 50% and 30% oxygen saturation, respectively, with a reduced frequency of feeding for plaice recorded at 30% oxygen saturation (Petersen and Pihl, 1995).

3.2.2.- Ammonia and urea excretion

Ammonia and urea are the two main excretory products of nitrogen metabolism in teleost fish (Handy and Poxton, 1993; Dosdat et al., 1996; Chadwick and Wright, 1999). Ammonia represents 75 to 90% and urea about 5 to 15% of total nitrogenous excreted by fish (Dosdat et al., 1996). The preferred form of expressing ammonia production and concentration is total ammonia as nitrogen (TAN) which includes both un-ionized ammonia as nitrogen (NH_3 – N) and ionized ammonium as nitrogen (NH_4^+ - N). Between the two compounds in total ammonia, un-ionized ammonia has been reported to be much more toxic than ionized ammonium (Colt and Armstrong, 1981; Meade, 1985). Metabolic nitrogen

compounds also may be excreted as urea (Brett and Zala, 1975; Randall and Wright, 1987).

The relationship between ingested nitrogen and TAN excretion is well documented in flatfish species (Jobling, 1981; Kikuchi et al., 1991; Dosdat et al., 1995; Carter and Bransden, 2001). High TAN excretion rates were evident in lemon sole, Atlantic halibut (*Hippoglossus hippoglossus*) and hirame 24 h after feeding (Davenport et al., 1990; Kikuchi et al., 1991). In hirame, 21 to 32% of the consumed nitrogen was excreted as TAN (Kikuchi, 1995). In turbot, TAN excretion was very low compared with the other species tested (sea bass, sea bream, brown trout, and rainbow trout), with 20% of ingested nitrogen instead of 30 to 38% in the others (Dosdat et al., 1996).

In some fish species, urea can make a substantial contribution to nitrogen excretion (Olson and Fromm, 1971; Walsh et al., 1990; Tanaka and Kadowaki, 1995; Kajimura et al., 2002). Urea excretion has proved to be an important component of nitrogenous excretion in flatfish species (Kikuchi et al., 1990; Kikuchi et al., 1991; Dosdat et al., 1995; Kikuchi, 1995; Dosdat el at., 1996; Carter et al., 1998; Verbeeten et al., 1999). A significant increase both in plasma urea-N levels and daily urea-N excretion rates was reported for juvenile turbot exposed to high ambient ammonia concentrations (Person-Le Ruyet et al., 1997; Person-Le Ruyet et al., 1998). Urea production was found to be of the same magnitude in turbot, sea bass, sea bream (*Sparus aurata*), brown trout (*Salmo*

trutta), and rainbow trout, for each class size (class size 10g and 100 g), representing 4 to 6% of ingested nitrogen when reared under optimum conditions (Dosdat et al., 1996). For turbot urea-N production amounted to 23% of the TAN and urea-N excretion, which was higher than for the other four fish tested by Dosdat et al. (1996). Urea production by other species, expressed as a percentage of the TAN and urea-N excretion, was 13% for Atlantic salmon (Fivelstad et al., 1990), and 10% in hirame (Kikuchi et al., 1992). It is known that urea in an aqueous media will be completely hydrolyzed to ionized ammonium and carbon dioxide in a few hours, if urea-hydrolizing bacteria are present (Pedersen et al., 1993), and hence it will become part of the TAN budget within the rearing unit (Kikuchi, 1995). Urease activity has been demonstrated in more than 200 species of bacteria, including both Gram-positive and Gram-negative (Pedersen et al., 1993).

Ammonia and urea excretion are not well correlated with fish swimming activity. A slow increase in the ammonia and urea excretion rate has been described for Nile tilapia (Alsop et al., 1999) as water velocity increases, but the rates were found to be independent of swimming velocity for rainbow trout (Alsop and Wood, 1997). However, swimming and resting rainbow trout tested for toxicity showed that the LC_{50} level decreased from 207.00 ± 21.99 mg TAN/L in resting fish to 32.38 ± 10.81 mg TAN/L in swimming fish (Randall and Tsui, 2002).

Toxic effects of TAN on fish physiology may include decrease of growth rate, diminished fertility and weakened immunity, as well as increased vulnerability to changes in temperature and oxygen levels (Handy and Poxton, 1993). For 3 g turbot fry, it was observed that wet mass decreased in a linear manner with increased concentrations of un-ionized ammonia when they passed the threshold concentration of 0.11 mg NH_3 – N/L (Alderson, 1979). Juvenile turbot (~20 g) reduced their food intake when un-ionized ammonia was over 0.117 mg NH_3 – N/L, and a reduction in body mass gain per day occurred when the level was over 0.108 mg NH_3 – N/L (pH 8, 16 °C, 28 g/L salinity) (Rasmussen and Korsgaard, 1996). Hence the threshold level seems not to exceed 0.11 mg NH_3 – N/L when optimal growth of turbot fry and juveniles is targeted. However later reports show that, in 13, 23, and 104 g turbot, the growth was not affected at concentrations of 0.21, 0.18, 0.09 mg NH_3 – N/L, respectively, while growth stopped immediately for all groups above 0.8 mg NH_3 – N/L (~pH 8, ~17 °C, 34.5 g/L salinity) reared in water having over 80% oxygen saturation (Person-Le Ruyet et al., 1997). A maximum level of 0.4 mg NH_3 – N/L (13 mg TAN/L) has been reported as not to affect turbot survival (Person-Le Ruyet et al., 1997). In sole and sea bream juveniles, the thresholds for no growth were between 0.38 – 0.77 (pH range 6.9 – 7.9) and 0.5 mg NH_3 – N/L, respectively (Alderson, 1979; Wajsbrot et al., 1993). Lethal concentrations (96-h LC_{50}) for juvenile marine species range from 1.7 to 2.7 mg NH_3 – N/L in sea bass, sea bream, and turbot (Wajsbrot et al., 1991; Person-Le Ruyet et al., 1995).

Large variations in the timing and the magnitude of peak nitrogen excretion have been reported for different fish (Brett and Zala, 1975; Rychly and Marina, 1977; Ramnarine et al., 1987; Dosdat et al., 1995: Kikuchi, 1995; Verbeeten et al., 1999; Bergero et al., 2001; Engin and Carter, 2001). Brett and Zala (1975) showed a TAN peak 4.5 h after feeding for sockeye salmon. Hirame fed once per day showed a maximum TAN peak 3 to 6 hours after feeding (Kikuchi, 1995). For greenback flounder (*Rhombosolea tapirina*), TAN peak excretion occurred 3 h after feeding, and was lower for morning than for evening fed fish (Verbeeten et al., 1999). In a study with juvenile Atlantic cod (*Gadus morhua*), peak excretion occurred 6.5 – 27 hours after feeding which depended on ration size and feeding frequency (Ramnarine et al., 1987). Rainbow trout fed twice a day at 8:00 and 17:00 h showed a peak for TAN excretion 6 h after being fed (Bergero et al., 2001). For juvenile turbot fed twice daily (10:00 and 16:00), two peaks were noticeable around 6 h post-feeding (Dosdat et al., 1995). Peaks of nitrogen excretion should be avoided, since they might reach toxic levels for the fish under culture (Thomas and Piedrahita, 1998). A general approach to avoid peaks of nitrogen excretion is by provide the daily feed ratio in several ratios within a day (Dosdat et al., 1995; Thomas and Piedrahita, 1998).

3.2.3.- Suspended solids

The main particulate wastes from marine recirculating finfish systems are produced in the fish culture tanks, ie. feces, uneaten feed, and fish mucus (Chen et al., 1993a; Patterson and Watts, 2003). It has been reported for salmonids that

approximately 1 kg of feed produces about 0.3 kg of fecal solids (Smith et al., 1980; Beveridge et al., 1991; Bergheim and Brinker, 2003; Patterson and Watts, 2003). A build up of solids in an aquaculture system can lead to problems with both the recirculating system components (Wheaton, 1977; McMillan et al., 2003) and the fish (Larmoyeux and Piper, 1973; McConnell, 1989; Noble and Summerfelt; 1996).

Suspended solids can cause a decline in water quality (Chen et al., 1993a, 1993b) that will create a variety of problems including, physiological stress on the culture organisms (Wedemeyer, 1996). Large quantities of suspended particulate matter may suffocate developing eggs during incubation, physically abrade or coat the gills (Wedemeyer, 1996), reduce dissolved oxygen levels as the solids decay (Cripps and Bergheim, 2000; Sumagaysay-Chavoso and San Diego-McGlone, 2003), and leach nutrients and toxic substances such as hydrogen sulfide (Wyban and Sweeny, 1989). Turbidity due to suspended solids may interfere with sight feeding fish species resulting in poor feed uptake (Timmons et al., 2001). Total suspended solids (TSS) and turbidity levels favoring optimum fish health are not yet known (Wedemeyer, 1996; Timmons et al., 2001).

3.3.- TANK DESIGN FOR FLATFISH

There are many types of fish rearing tanks in use, each with a spectrum of advantages and disadvantages (Timmons et al., 2001). A given tank is chosen

for reasons of costs, utilization of space, and various fish rearing considerations. Major fish culture variables to be considered include water flow, water velocity, velocity distribution, sedimentation and cleaning, and water exchange, which will depend upon the number of fish stocked within the culture unit and feeding rates. Guidelines for tank design have been widely reported for a variety of species (Timmons and Young, 1991; Pennel and McLean, 1996; Timmons et al., 1998; Timmons et al., 2001). In general, the most suitable design for a flatfish tank appears to be circular, because of its more even flow distribution and better self-cleaning in restricted flow conditions, compared with other designs (Cripps and Poxton, 1992). However, rectangular tanks are more efficient in terms of fish farm floor surface areas than circular tanks (Kerr, 1981; Klapsis and Burley, 1984). The Burrows pond, a rectangular D ended raceway (Burrows and Chenoweth, 1970) looks particularly suitable for flatfish culture, since it has both circular and rectangular tank properties (Cripps and Poxton, 1992). In addition shallow tanks will be more suitable for flatfish species, due to their benthic behavior (Cripps and Poxton, 1993).

3.3.1.- Stocking density

One of the first issues to be answered in designing a RAS is the number of fish that can be reared in a tank. The number of fish and their mass will define the feeding rates from which the required bioengineering parameters will be defined, and from here the RAS and its water treatment devices can be designed. The number of fish that can be stocked per unit of volume or surface area will depend

both on the fish species and fish size. Flatfish species such as hirame (Jeon et al., 1993), turbot (Martinez-Tapia and Fernandez-Pato, 1991; Irwin et al., 1999; Irwin et al., 2003), Atlantic halibut (Bjornsson, 1994), and summer flounder (*Paralichtys dentatus*) (King et al., 1998) have shown a direct correlation between stocking density and growth rate.

Hirame juveniles of about 1 to 5 g wet mass are stocked into the culture tank (4 to 10 m^2) at about 100 to 800 fish/m^2 (Kikuchi, 2000; Seikai, 2000). Culture densities for 1 kg fish are less than 15 kg/m^2 in tank sizes ranging from 30 to 100 m^2 (Kikuchi, 2000; Sikai, 2000). Overstocking decreases growth and feed efficiency. Recommended stocking densities for hirame are presented in Table 3.1.

Turbot juveniles withstand initial stocking density of 2500 fish/m^2 for a 100 mg fish (Person Le-Ruyet et al., 1983). At this stage, turbot juveniles are stocked in large square shallow tanks of about 0.4 to 12 m^3 (Silva and Velez, 1998). The stocking biomass may progress from 2 kg/m^2 to 12 kg/m^2 (Table 3.2).

Table 3.1.- Standard stocking densities in landbased tanks for hirame (Seikai, 2000).

Total length (cm)	Body mass (g)	Stocking (fish/m^2)	Stocking (kg/m^2)
5	1.5	800	1.2
10	10	200	2
15	60	95	5.7
20	85	50	4.3
25	140	35	4.9
30	320	22	7
35	460	17	7.8
40	800	13	10.4

Table 3.2.- Stocking densities for turbot reared at 16 to 18 °C (Person Le-Ruyet et al., 1983).

Wet fish mass (g)	Age (months)	Stocking Fish/m^2
0.1	1	2500
2	3	1000
10	5	500
35	7	250
75	9	150
125	11	100

Turbot can tolerate culture densities equivalent to 75 to 120 kg/m^2 (Person Le-Ruyet et al, 1983; Liewes, 1983). However, Lygren (1993) using a modified raceway with shelves and a water depth of 2.55 m, increased the density to 175-200 kg/m^2 in a commercial farm. However, the normal operational range varies from 25 to 50 kg/m^2 depending on environmental rearing conditions (Jones, 1981; Person Le-Ruyet et al., 1983; Liewes, 1984; Silva and Velez, 1998).

3.3.2.- Water velocity

High stocking densities in terms of biomass are often limited by the rate of water flow through the rearing unit (Westers and Pratt, 1977; Fivelstad, 1988). Water flow rates should be sufficient to ensure adequate oxygen availability (Lawson, 1995). However these flow rates may result in higher than desired water velocities, and may negatively affect fish growth rates. Water velocity can significantly affect fish performance in culture tanks as has been shown for brook trout (*Salvelinus fontinalis*) (Leon, 1986), ayu (*Plecoglossus altivelis*) (Nakagawa et al., 1991), channel catfish (*Ictalurus punctatus*) (Jarboe and Grantt, 1996), red sea bream (*Pagrus major*) (Forster and Ogata, 1996), and hirame (Ogata and Oku, 2000). Water flow velocities can be related to fish size (total length, TL) through the estimation of a relative swimming velocity that is expressed as body lengths per second (bl/s) (Hammer, 1995).

Ogata and Oku (2000) were the first to study the relationship between water velocity and growth in flatfish species, hirame, for aquaculture purposes. They

found that water velocities greater than 2.1 bl/s (19.5 to 23.5 °C) led to growth retardation and lower feed efficiency. Ogata and Oku (2000) also found that the optimal water velocity for hirame growth occurred at about 1.0 bl/s for a 12.5 cm/body length (5.7 g/fish).

Presently, no research has been conducted which provides a direct comparison of California halibut performance over a range of water velocities. There are, however, studies that have addressed the growth of California halibut cultured in an environment where water flow has been established. Oiestad (1999) stocked pre-metamorphic California halibut (7-8 mm and 8-10 mg mass) in shallow raceways yielding a 20% specific growth rate (SGR) during the first week when offered enriched *Artemia* nauplii, and a SGR 12-15% with a diet of frozen artemia (~100 mg mass). Jirsa and Drawbridge (2000) reared methamorphosed California halibut up to 4.3 g (28% survival, ~235% coverage area, ~6.3 kg/m^2) in shallow raceways with current velocities starting at 0.3 cm/s and ending with 22 cm/s (~2.75 bl/s). Innis (1980) grew juvenile (16 to 24 cm TL) and subadult (> 25 cm TL) California halibut in raceways (245 x 110 x 76 cm) for 256 days. However, none of their studies targeted an understanding of the water velocity which must be chosen for California halibut culture or whether there is any effect on growth rates and survival. Effects of water flow velocities on growth for California halibut should be addressed to provide with basic information which might be practical in the design of an artificial environment for farming this species.

Relative swimming velocity information is important in the design of a flatfish culture tank (Cripps and Poxton, 1992) because it will affect the operation in terms of water velocity, velocity distribution, sedimentation and cleaning, and flushing or water exchange (Varadi, 1984; Pennell and McClean, 1996). Raceways are said to be self-cleaning if water velocities along the bottom are greater than 6 cm/s (Burrows and Chenoweth, 1970; Timmons and Young, 1991).

3.4.- MASS BALANCES

Determination of bioengineering parameters and predictability of water quality changes are essential for the design of intensive RAS for fish, as reported by Honda and Kikuchi (1995) for hirame. Without the knowledge of bioengineering parameters, the process for determining a reliable flow rate and quality of incoming water, required for establishing suitable living conditions for a fish, is quite difficult (Liao, 1971; Westers and Pratt, 1977; Fivelstad, 1988).

The mass balance approach has been used to solve aquacultural engineering problems related to any variable affecting water quality (Liao and Mayo, 1972; Losordo and Westers, 1994; Eikebrokk and Piedrahita, 1997; Timmons et al, 2001). A mass balance equation for a given variable affecting water quality applied to a control volume (Fig. 3.1) can be described in words as:

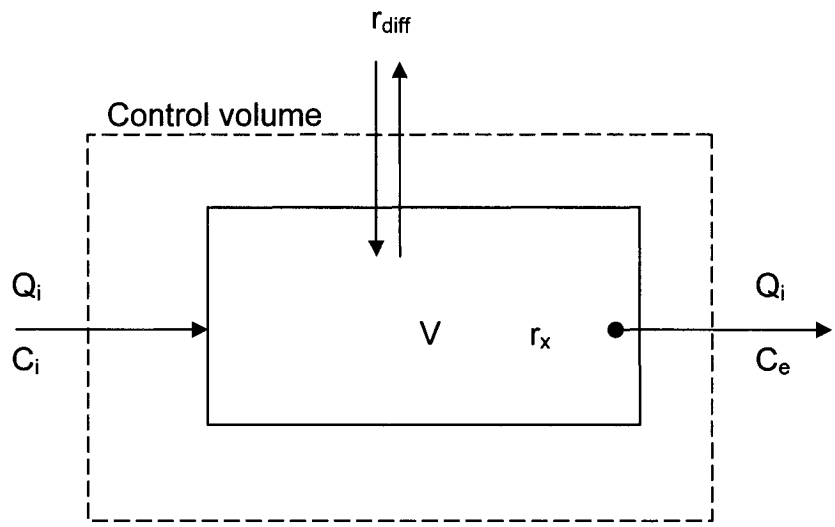

Figure 3.1- General mass balance on a fish culture tank. Terms are as defined in the text.

Accumulation = Input – Output + Generation – Consumption (Eq. 3.1)

The *accumulation* term is equal to zero if the compound of interest has reached steady state conditions. The *input/output* terms are defined by the fluxes for the compound of interest. The *generation/consumption* terms are usually related proportionally to the fish feeding rate (Blancheton, 2000; Timmons et al., 2001). The words in the above mass balance equation (Eq. 3.1) can be turned into a differential equation (Thomas and Piedrahita, 1997) for a system of constant volume:

$$\frac{dC}{dt}V = Q_i C_i - QC_e + r_x V \qquad \text{(Eq. 3.2)}$$

where, dC/dt = rate of change in concentration of the reference material in the tank (mg / L h)

V = tank volume (L)

Q_i = flow rate of water entering the tank (L/h)

C_i = concentration of the reference material in the influent flow (mg / L)

C_e = concentration of the reference material in the effluent flow (mg /L)

r_x = net rate of production of the reference material (mg / L h). A negative r_x stands for a net consumption of the reference material.

3.4.1.- Laboratory versus field studies

Metabolism studies on flatfish in laboratory have been addressed with the use of respirometry chambers (Klein-MacPhee, 1979; Wood et al., 1979; Priede and Holyday, 1980; Honda, 1988; Winger et al., 1999). However it has been demonstrated that there are large differences between the metabolic rates determined in the laboratory and field data determined at a fish farm (Muller-Feuga et al., 1978; Bergheim et al., 1993; Fivelstad et al., 1999). Honda (1988) reported that oxygen consumption in hirame held singly was 11 to 17% greater than that of fish held in groups. Hence studies of metabolic rates with single fish may overestimate the real needs of a given compound.

Oxygen consumption rates of fish species under farming conditions have been estimated using culture tanks as open respirometers (Brown et al., 1984; Hallaraker et al., 1995; Imsland et al., 1995; Thomas and Piedrahita, 1997; Fivelstad et al., 1999). Steffensen (1989) stated that the effectiveness of open respirometers is limited, due to a lag in changes in oxygen consumption behind a change in activity, which depends on the effective mixing in the respirometer, the volume of the respirometers, and the water flow. Furthermore there is interaction with the atmosphere, and surface oxygen diffusion will occur in the tanks (Thomas and Piedrahita, 1997; Fivelstad et al., 1999). Surface oxygen diffusion is likely to be driven from the atmosphere towards the water in the culture tanks, due to fish respiration and the resultant reduction of dissolved oxygen. Biofouling growing on tank walls (Tudor, 1999), tank shape and farm scale (Bergheim et al.,

1993), fish activity and feeding level (Fivelstad et al., 1999), as well as stress associated with daily husbandry may also be important parameters contributing to the total oxygen requirements in a fish farm. Oxygen consumption rates, or any metabolic variable, determined under farming conditions will account for the everyday effects of management on fish metabolism.

3.5.- CONCLUSIONS

California halibut is a gregarious species in the wild and when stocked in tanks, high stocking densities might be possible in a commercial aquaculture system (James Rounds, Calif. Halibut Hatchery Program. Personal communication, 2001). The ability to raise California halibut at a relatively high density, thus maximizing culture area usage, is of particular importance for future commercial operations.

High fish stocking densities will need larger water flow supplies than low stocking densities. Water is the media through which fish get oxygen and through which they release their metabolites. Water velocities within a rearing tank can be expected to reach unacceptable levels when rearing fish at high density. The magnitude of water velocity will depend upon tank configuration, fish stocking density, and fish swimming performance.

Flatfish species can be raised at high densities when adequate flow and water quality are maintained (Martinez-Tapia and Fernandez-Pato, 1991; Bjornsson, 1994; King et al., 1998; Malleck et al., 1998). However, there are no studies for California halibut regarding adequate levels of dissolved oxygen, TAN, and suspended solids applicable for their culture.

Hence, studies are needed to approach the design of a reliable culture system for California halibut. These studies will allow to approach sizing for a culture tank to rear California halibut as well as to advise for an adequate stocking density, water velocity, and to select and size the unit operations which could be added to the culture system for water treatment purposes.

CHAPTER IV

EFFECT OF WATER VELOCITY ON THE GROWTH OF CALIFORNIA HALIBUT JUVENILES

4.1.- INTRODUCTION

There are many unknowns with respect to dealing with a new species potentially suitable for aquaculture, such as California halibut (*Paralichthys californicus*). Among the unknowns, usually flow rate and the quality of incoming water are the first parameters to be dealt with in establishing suitable living conditions for a fish (Liao, 1971; Westers and Pratt, 1977; Fivelstad, 1988). Usually oxygen is the limiting factor among the water quality variables, and therefore it is the basis for determining water flow requirements for sustaining stock (Elliot, 1969; Brown et al., 1984; Bergheim et al., 1993). High stocking densities generally require high water flow rates into the tanks to supply the oxygen and to carry out the metabolic byproducts. A factor closely related to flow rate is current velocity, which can significantly affect fish performance in culture tanks, as has been shown for brook trout (Leon, 1986), ayu (Nakagawa et al., 1991), channel catfish (Jarboe and Grantt, 1996), red sea bream (Forster and Ogata, 1996), and hirame (Ogata and Oku, 2000). Water velocities tolerated by fish are species specific and positively dependent on fish size and water temperature (Bainbridge, 1960; Brett and Glass, 1973; Duthie, 1982; He and Wardle, 1988; Winger et al., 1999).

Presently, no research has been conducted which provides a direct comparison of California halibut performance over a range of water velocities. There are, however, studies that have addressed the growth of California halibut cultured in an environment where water flow has been established: Oiestad (1999) stocked pre-metamorphic California halibut (7-8 mm and 8-10 mg mass) in shallow raceways (no mention of water velocity); Jirsa and Drawbridge (2000) reared methamorphosed California halibut up to 4.3 g in shallow raceways with current velocities starting at 0.3 cm/s and ending with 22 cm/s; Innis (1980) grew juvenile (16 to 24 cm TL) and subadults (> 25 cm TL) California halibut in raceways (245 x 110 x 76 cm) for 256 days (water level not mentioned). However, none of their studies attempted an understanding of the water velocity that must be chosen for California halibut culture or whether there is any effect of water velocity on growth rates and survival. Since no data are currently available on the effects of water flow velocities on growth for California halibut juveniles, an experiment was designed to provide this information.

4.2.- LITERATURE REVIEW

Water flow velocities have been suspected to affect growth and behavior in a variety of farmed flatfish species: turbot (Liewes, 1984; Oiestad, 1995; Klokseth and Oiestad, 1999a), sole (Liewes, 1984), and Atlantic halibut (Oiestad, 1995; Klokseth and Oiestad, 1999b). Liewes (1984) observed that "high water

velocities" (no value given) are beneficial for turbot culture but not suitable for sole culture; however the author could not explain this difference.

Laboratory studies have shown a positive dependency of fish size and water temperature on swimming velocity in flatfish species such as winter flounder (Beamish, 1966), American plaice (*Hippoglossoides platessoides*) (Winger et al., 1999), hirame (Hiraishi et al., 1995; Hashimoto et al., 1996), and longsnout flounder (*Limanda punctatissima*) (Hiraishi et al., 1995). Water flow velocities can be related to fish size (total length, TL) through the estimation of a relative swimming velocity that is expressed as body lengths per second (bl/s) (Hammer, 1995).

A number of long-term studies have shown that fish growth is promoted when fish are reared at moderate swimming velocities (Davison and Goldspink, 1977; Totland et al., 1987; Christiansen and Jobling, 1990; Jarboe and Grant, 1996). However, in other studies, both growth rate and feed conversion ratio have been found to be negatively related to swimming velocity (Forster and Ogata, 1996; Kiessling et al., 1994; Davison and Goldspink, 1978). Among flatfish species, relative swimming velocity effects on growth have been quantified under farm-like conditions only for hirame (Ogata and Oku, 2000) and summer flounder (Bengston and Alves, 2002). Ogata and Oku (2000) found that water flow velocities that imply a relative swimming velocity beyond 2.1 bl/s (19.5 to 23.5 °C) led to growth retardation and lower feed efficiency for all hirame sizes tested.

Ogata and Oku (2000) also found that the optimal water flow velocity for hirame growth occurred at about 1.0 bl/s for a 12.5 cm/body length (5.7 g/fish). Preliminary results with two sizes of summer flounder (124 and 387 g average mass) have shown that survival was affected negatively at the highest water velocity tested (30 cm/s; ~1.55 bl/s for 124 g fish; ~1.06 bl/s for 387 g fish), and growth was better with the medium velocity tested (15 cm/s; ~0.77 bl/s for 124 g fish; ~0.53 bl/s for 387 g fish) (Bengston and Alves, 2002). Ogata and Oku (2000) described a constant waving of the posterior fin in Japanese flounder held at 1.0 and 2.1 bl/s relative swimming velocities. Arnold (1969) observed in laboratory studies that plaice held at a relative swimming velocity of ~1.5 bl/s increased the beating of the posterior fin. The beating of the posterior fin was suggested as a mechanism to maintain position on the bottom against the water current (Arnold, 1969; Arnold and Weihs, 1978; Ogata and Oku, 2000).

4.3.- EXPERIMENTAL DESIGN

4.3.1.- Fish stock

Weaned California halibut 90 days post hatch (dph) were used for this study. The fish were grown in the California Halibut Experimental Recirculating Hatchery, located at the University of California, Davis (Appendix A.1). The juveniles (1.53 g average wet mass) were reared in raceways within a recirculating seawater system, at a constant temperature of 21±1 °C and salinity of 30±2 g/L (Gadomski and Caddell, 1991; Madon, 2002). The fish were fed daily at ~7% body mass with

commercial dry pelleted feeds (BioKyowa™ 1000). Fish feeding was suspended 24 h prior to their transfer to experimental tanks. Light was provided by overhead fluorescent tubes on a 16 L: 8 D (L=light; D=dark) photoperiod (Boeuf and Le Bail, 1999; Klokseth and Oiestad, 1999a).

4.3.2.- Experimental tank

Rectangular tanks were used in this experiment to provide the velocities to be tested (Fig. 4.1). The tanks were 60 cm in total internal length. Three tank widths were used to achieve the desired water velocities: 9.3 cm, 12.3 cm, and 15.3 cm. Screens were placed at both ends of the tank to hold the fish within the experimental area. The culture area was increased during the last two weeks of the experiment to maintain a culture density lower than 150% of coverage area (Table 4.1). The culture area was increased by movement of the influent screen. The water depth in each tank was controlled by a weir (Fig. 4.2).

Tanks were supplied with seawater from the recirculating system (Appendix A.1). A manifold built from 2.54 cm nominal diameter PVC pipe with nine valves distributed the required amount of seawater to the head of each experimental tank. Effluent water from all tanks flowed to a settling tank and then, by gravity, to the head of the biofilter.

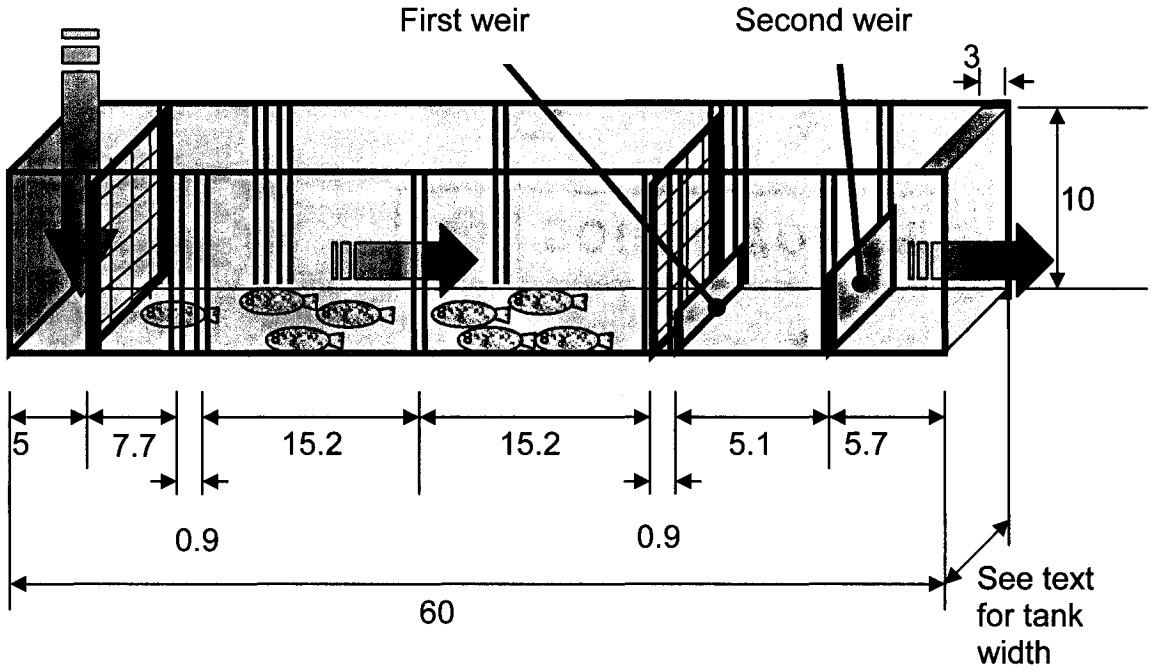

Figure 4.1. Schematic diagram of a tank used for the velocity experiment. All dimensions are shown in cm. Each slot to introduce nets or weirs is 0.6 cm wide (measurement not shown). Figure is not to scale. Large arrow indicates the direction of the water flow. Tanks width were 9.3, 12.3, and 15.3 cm. Water depth is not shown, however it was between 1.2 and 1.4 cm. Tanks were built from dark grey acrylic glass, 0.5 cm thick.

Table 4.1.- Tank dimensions and operating conditions during the ten week growth trial.

	Tank data during the first eight weeks								
Tank	1	2	3	4	5	6	7	8	9
Width (cm)	12.3	12.3	12.3	9.3	9.3	9.3	15.3	15.3	15.3
Depth (cm)	1.4	1.3	1.4	1.4	1.3	1.3	1.2	1.2	1.3
Length (cm)	32.5	32.5	32.5	32.5	32.5	32.5	32.5	32.5	32.5
Surface (cm^2)	399.75	399.75	399.75	302.25	302.25	302.25	497.25	497.25	497.25

	Tank data during weeks nine and ten								
Tank	1	2	3	4	5	6	7	8	9
Width (cm)	12.3	12.3	12.3	9.3	9.3	9.3	15.3	15.3	15.3
Depth (cm)	1.4	1.3	1.4	1.4	1.3	1.3	1.2	1.2	1.3
Length (cm)	42	42	42	42	42	42	42	42	42
Surface (cm^2)	516.6	516.6	516.6	390.6	390.6	390.6	642.6	642.6	642.6

Length and surface dimensions are for the section where the fish were enclosed.

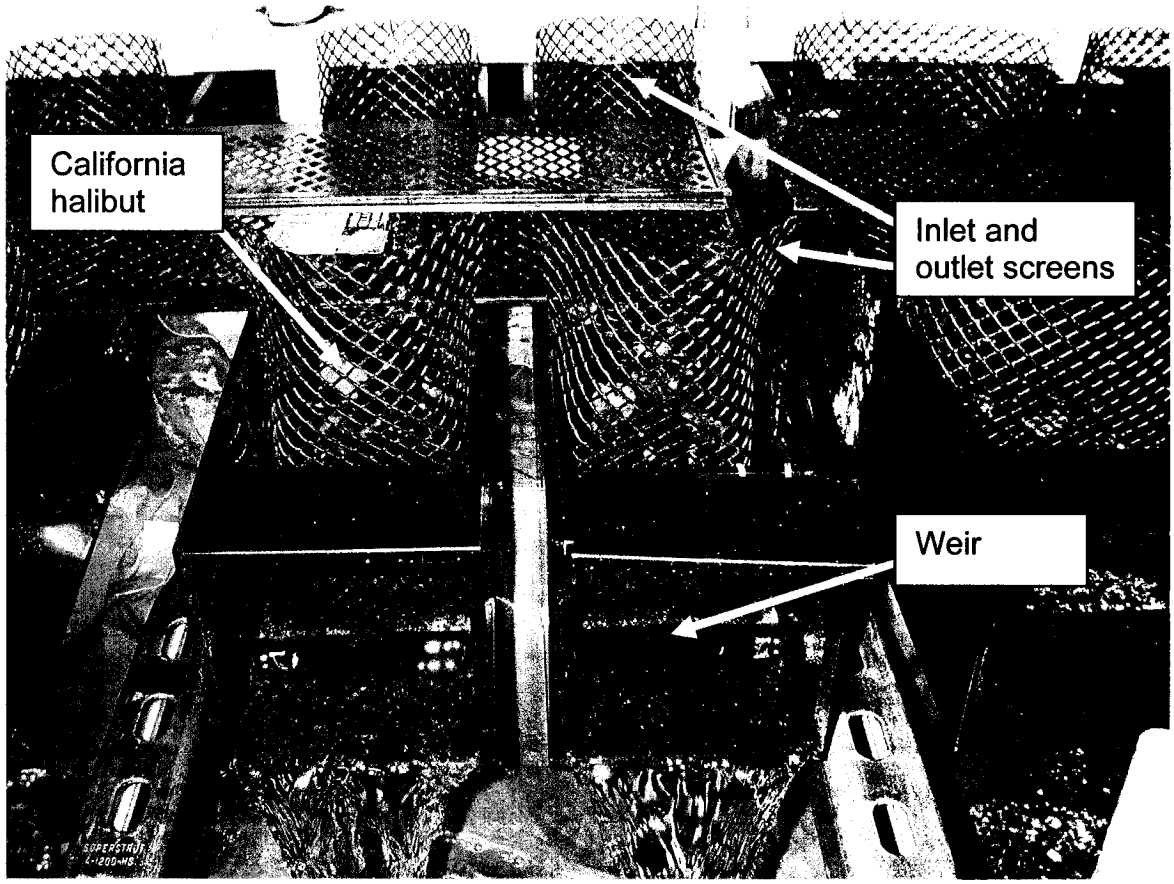

Figure 4.2.- Detailed view of experimental tank set up.

4.3.3.- Culture protocol

Nine tanks were stocked with 15 fish each (Fig. 4.3), and the fish growth was followed over 10 weeks. The fish were fed commercial dry pelleted feeds (BioKyowa™ 1000, Silver Cup™ 1000, EWOS™ 1 and 2 mm) by hand twice a day (09:00 and 16:00 h) for 10 weeks. Pelleted feed was changed during the experiment according to fish size. Fish were fed at the rate of 7% body mass per day (% BW/d) for weeks 1 and 2, 5% BW/d for weeks 3 to 8, and 3.8% BW/d for weeks 9 to 10.

Each tank was supplied with two water lines (Fig. 4.4). A primary water line was used to deliver the required flow to achieve a given water velocity into each tank. A secondary water line was used to supply a reduced water flow during the fish feeding process. During feeding, the primary water line was turned off (inlet hoses were removed from each tank) and the secondary water line was turned on for 30 minutes; at the same time water depth was increased up to 6 cm in all raceways by increasing the height of the weirs.

Water flow reduction during feeding allowed the fish in each tank to have equal time for accessing the feed. Immediately after feeding was completed, the primary water line was turned on by putting inlet hoses back in each tanks, the secondary flow was turned off, and the water depth was restored to its original level. Reducing the water level from 6 to between 1.2 cm and 1.4 cm created a flushing effect that helped to remove any uneaten pellets from the tanks. Any dead fish were removed and not replaced.

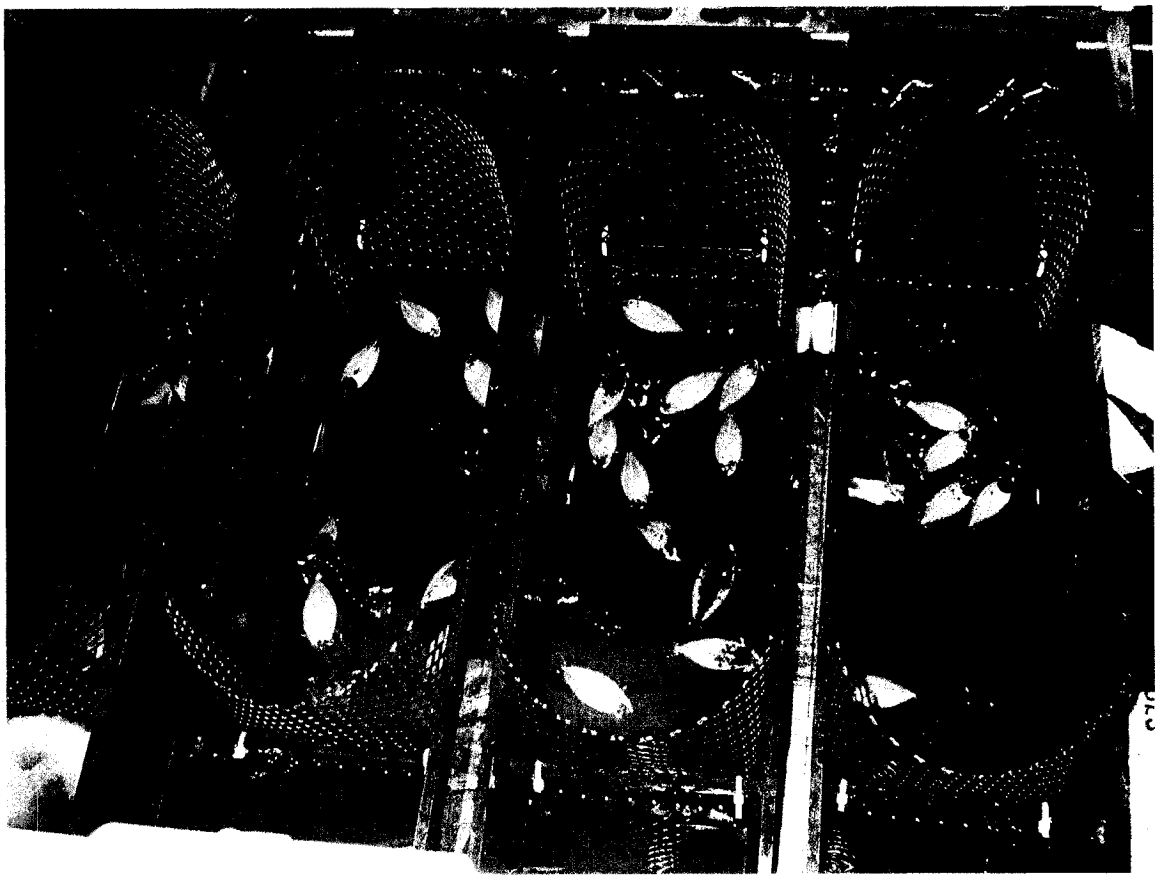

Figure 4.3.- Set up of triplicate tanks for 1.0 bl/s treatment.

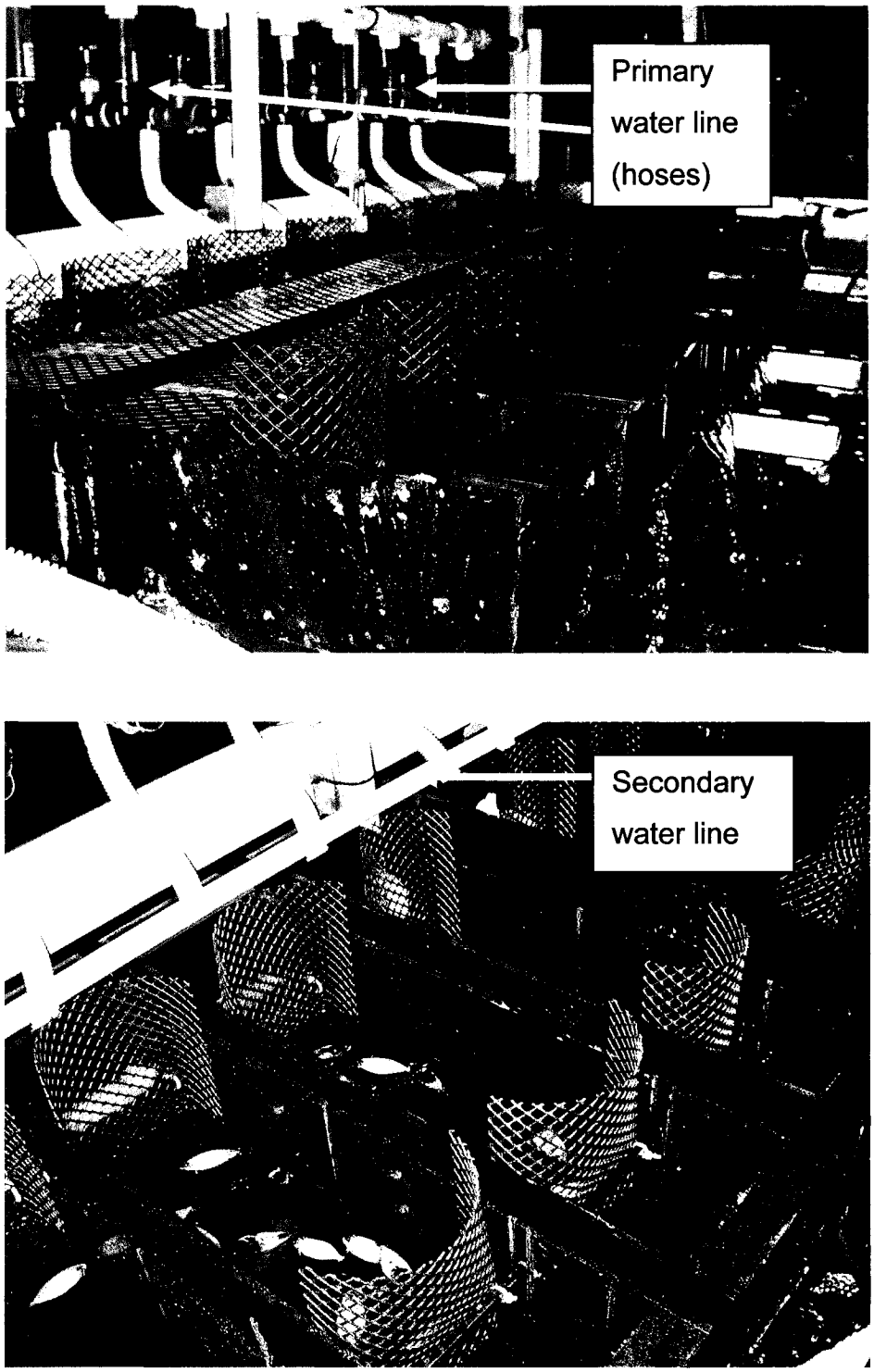

Figure 4.4.- Tank set up with California halibut juveniles. Primary and secondary distribution pipe are shown.

4.3.4.- Measurements of water velocity and water quality

Average water velocities within the tanks were adjusted every other week using the average total length of the fish within the respective experimental tank. The water flow required for each experimental raceway was estimated from the relationship between average water velocity and tank cross sectional area. Water flow rates were determined from measurements of the time required to fill a known volume. Water flow rates were estimated in triplicate per each experimental raceway.

Dissolved oxygen (DO) concentrations were recorded every 10 min with a dissolved oxygen sensor (Sensorex model DO6000) located in the head tank of the recirculating system (Appendix A.1). Experimental tank dimensions did not allow for the installation of a DO sensor for continuous monitoring of influent concentration. The influent concentration was measured over a period of time and it was found to be 0.69 ± 0.04 mg DO/L lower than the concentration in the head tank, which was monitored continuously. Hence, DO monitored at the head tank was reduced by 0.69 to estimate levels of oxygen supplied to the head of the experimental tanks. The recirculating water temperature was monitored with a copper-constantan thermocouple. Data from the oxygen sensor and thermocouple were recorded with a micrologger (21X, Campbell Scientific, Inc.).

4.3.5.- Fish sampling

All fish were sampled every other week to record their biomass and morphometric data. Morphometric data were collected by image analysis (Appendix A.2). Fish were unfed 24 h before and on the sampling day. Feeding was resumed the day after sampling. The parameters that were measured were total body length (TL, cm), fish average biomass (W, g), body surface area (BSA, cm^2), and fish total surface area (TSA, cm^2) (Appendix A.2). The following parameters were estimated:

a) Culture density as percentage of coverage area (PCA, %)

$$PCA = 100 * (TSA * N) / A \qquad \text{(Eq. 4.1)}$$

where TSA = average total surface area (cm^2)

N = number of fish under culture

A = tank surface area used for culture (cm^2).

b) Biomass gain (BG, g):

$$BG = B_{END} - B_{START} \qquad \text{(Eq. 4.2)}$$

where B_{END} = total biomass at sampling time (g)

B_{START} = total biomass at stocking time (g)

c) Coefficient of variation for mass (CV, %):

$$CV = (SD * 100) / W \qquad \text{(Eq. 4.3)}$$

CV was assessed to determine fish size variation within the culture tanks (SD stands for standard deviation).

d) Percentage of growth gain:

$$PWG = (W_{END} - W_{START}) \times 100 / W_{START} \qquad \text{(Eq. 4.4)}$$

$$TSAG = (TSA_{END} - TSA_{START}) \times 100 / TSA_{START} \qquad \text{(Eq. 4.5)}$$

$$BSAG = (BSA_{END} - BSA_{START}) \times 100 / BSA_{START} \qquad \text{(Eq. 4.6)}$$

where PWG = percentage of average mass gain (%)

W_{END} = average wet biomass at sampling time (g)

W_{START} = average wet biomass at stocking time (g)

TSAG = total surface area gain (%)

TSA_{END} = average total surface area at sampling time (cm^2)

TSA_{START}= average total surface area at stocking time (cm^2)

BSAG = body surface area gain (%)

BSA_{END} = average body surface area at sampling time (cm^2)

BSA_{START}= average body surface area at stocking time (cm^2)

e) Specific growth rate as mass (SGR, %/day), calculated as percent of body mass gain per day (Fonds et al., 1995):

$$SGR = 100 * (\ln B_{END} - \ln B_{START}) / (t_{END} - t_{START}) \qquad \text{(Eq. 4.7)}$$

where t_{END} = final time (days)

t_{START} = initial time (days).

f) Feed efficiency (FE, g/g) (Forster and Ogata, 1996):

$$FE = BG / F \qquad \text{(Eq. 4.8)}$$

where F = feed offered (g).

g) Relative swimming velocity (RSP, bl/s):

$$RSP = V_{WATER} / TL \qquad \text{(Eq. 4.9)}$$

where V_{WATER} = water flow velocity within the vessel (cm/s)

TL = average fish total length (cm/bl).

4.3.6.- Experimental design and statistical analysis

The experiment was designed as a one-factor study in which the effects of three water flow velocities on California halibut growth were examined after ten weeks of culture. The treatment factor water velocity was a fixed effect, since its levels were specifically chosen. Water velocity levels were specified as relative swimming velocities of 0.5, 1.0, and 1.5 bl/s. The experimental unit to which velocity treatments were applied was California halibut stocked within the tanks. California halibut juveniles (135 fish with 1.53 ± 0.12 g average wet mass) were randomly divided (15 fish/tank) into three treatment groups with three replicates per treatment.

Fish total population within each tank was sampled every other week for estimates of the response variables. A one-way complete model analysis of variance (Dean and Voss, 1999) was performed with SAS statistical software to analyze the response variables W, TL, TSA, BSA, SGR, PWG, TSAG, BSAG, BG, and FE among treatment levels for Week 0 and Week 10. The stated null hypothesis (H_o) was that there is no difference in fish growth between water flow velocity levels, and the corresponding alternative hypothesis (H_i) was that at least one level is different. Significances were analyzed with Duncan's multiple range test and Tukey's studentized range test at a significance of 0.05 (Dean and Voss, 1999).

Every other week response variables W, SGR, and CV were analyzed for significant differences among treatments through repeated-measures analysis of variance (ANOVA) with SAS statistical software. The stated null hypothesis (H_o) was that there is no difference in response variables among treatment levels every other week, and the corresponding alternative hypothesis (H_i) was that at least one level is different. Differences among variables were analyzed with Duncan's multiple range test and Tukey's studentized range test at a significance of 0.05 (Dean and Voss, 1999).

4.4.- RESULTS

There were no mortalities during the ten week experimental trial. During Week 8 one fish jumped out from one tank of treatment 1.5 bl/s and another from the tank of treatment 0.5 bl/s. These fish were found but not returned to the treatments. During the growth trial, all fish were resting on the bottom of the tank at the velocities tested, meaning that the water velocities were not sufficient to carry them towards the effluent net. However, fish kept at 1.0 and 1.5 bl/s showed a constant waving of the posterior fin. During the sampling process, fish reared at 1.5 bl/s attached themselves strongly to the bottom of the sampling chamber.

4.4.1.- Water quality

Water quality variables were stable during the ten week experiment with a salinity of 30.7 ± 0.9 g/L; a pH of 7.67±0.25; an alkalinity of 2.7±0.4 meq/L; a TAN of

0.06 ± 0.05 g/L and nitrite-N of 0.54 ± 0.41 g/L. Estimated levels of influent dissolved oxygen were 6.76 ± 0.15 mg/L. Water temperature was 21.9 ± 0.2 °C.

TAN and nitrite-N were less than 0.13 and 0.8 mg/L, respectively, during most of the experimental time. However, during Week 8, both TAN and nitrite-N increased up to ~1.8 and ~1.5 mg/L, respectively, and then returned to the values observed in preceding weeks by Weeks 9 and 10.

4.4.2.- Analysis of fish growth between Week 0 and 10

Water flow velocities were adjusted every other week after the fish had been sampled for TL. Tables 4.2 - 4.6 summarize the values used to estimate the required water flow per tank. After fish sampling, PCA was also determined. To keep the PCA under 150% the culture area within each tank was adjusted on Week 9 (Tables 4.7 - 4.12).

Table 4.13 list fish mass and morphometric data at the beginning and end of the experiment. A slight decrease in the values of morphometric data for fish reared at the highest (1.5 bl/s) in comparison to the lowest (0.5 bl/s) water flow velocities was observed. Significant differences ($p < 0.05$) were found among the three levels of water flow velocities tested for W, TL, TSA, and BSA (Table 4.13). California halibut reared at 0.5 bl/s were larger than those reared at 1.5 bl/s, but similar to the ones held at 1 bl/s.

Table 4.2.- Flow required for each raceway at the beginning of Week 1 based on the average of total fish length within a given raceway and the targeted relative swimming velocity (RSP).

	(a)	(b)	(c) =(a) * (b)	(d)	(e)	(f) = (d) * (e)	(g) = (c) * (f)	
Experimental unit #	RSP	Average total fish length	Water velocity	Tank dimensions width	depth	cross area	Water flow required	
	bl/s	cm	cm/s	cm	cm	cm^2	cm^3/s	L/min
Raceway 1	1	5.21	5.21	12.3	1.4	17.22	89.76	5.39
Raceway 2	1	5.40	5.40	12.3	1.3	15.99	86.28	5.18
Raceway 3	1	5.32	5.32	12.3	1.4	17.22	91.67	5.50
Raceway 4	1.5	5.31	7.96	9.3	1.4	13.02	103.68	6.22
Raceway 5	1.5	5.56	8.34	9.3	1.3	12.09	100.87	6.05
Raceway 6	1.5	5.22	7.83	9.3	1.3	12.09	94.60	5.68
Raceway 7	0.5	5.39	2.69	15.3	1.2	18.36	49.47	2.97
Raceway 8	0.5	5.57	2.79	15.3	1.2	18.36	51.17	3.07
Raceway 9	0.5	5.35	2.67	15.3	1.3	19.89	53.17	3.19

Table 4.3.- Flow required for each raceway at the beginning of Week 3 based on the average of total fish length within a given raceway and the targeted relative swimming velocity (RSP).

	(a)	(b)	(c) =(a) * (b)	(d)	(e)	(f) = (d) * (e)	(g) = (c) * (f)	
Experimental unit #	RSP	Average total fish length	Water velocity	Tank dimensions width	depth	cross area	Water flow required	
	bl/s	cm	cm/s	cm	cm	cm^2	cm^3/s	L/min
Raceway 1	1	6.83	6.83	12.3	1.4	17.22	117.62	7.06
Raceway 2	1	6.73	6.73	12.3	1.3	15.99	107.58	6.45
Raceway 3	1	6.82	6.82	12.3	1.4	17.22	117.45	7.05
Raceway 4	1.5	6.87	10.30	9.3	1.4	13.02	134.13	8.05
Raceway 5	1.5	6.94	10.41	9.3	1.3	12.09	125.86	7.55
Raceway 6	1.5	6.43	9.65	9.3	1.3	12.09	116.68	7.00
Raceway 7	0.5	6.88	3.44	15.3	1.2	18.36	63.14	3.79
Raceway 8	0.5	7.22	3.61	15.3	1.2	18.36	66.27	3.98
Raceway 9	0.5	6.98	3.49	15.3	1.3	19.89	69.43	4.17

Table 4.4.- Flow required for each raceway at the beginning of Week 5 based on the average of total fish length within a given raceway and the targeted relative swimming velocity (RSP).

	(a)	(b)	(c) =(a) * (b)	(d)	(e)	(f) = (d) * (e)	(g) = (c) * (f)	
Experimental unit #	RSP	Average total fish length	Water velocity	Tank dimensions width	depth	cross area	Water flow required	
	bl/s	cm	cm/s	cm	cm	cm^2	cm^3/s	L/min
Raceway 1	1	8.32	8.32	12.3	1.4	17.22	143.26	8.60
Raceway 2	1	8.23	8.23	12.3	1.3	15.99	131.65	7.90
Raceway 3	1	8.24	8.24	12.3	1.4	17.22	141.97	8.52
Raceway 4	1.5	8.19	12.29	9.3	1.4	13.02	160.00	9.60
Raceway 5	1.5	8.22	12.33	9.3	1.3	12.09	149.02	8.94
Raceway 6	1.5	7.64	11.45	9.3	1.3	12.09	138.48	8.31
Raceway 7	0.5	8.10	4.05	15.3	1.2	18.36	74.38	4.46
Raceway 8	0.5	8.71	4.36	15.3	1.2	18.36	79.96	4.80
Raceway 9	0.5	8.50	4.25	15.3	1.3	19.89	84.57	5.07

Table 4.5.- Flow required for each raceway at the beginning of Week 7 based on the average of total fish length within a given raceway and the targeted relative swimming velocity (RSP).

	(a)	(b)	(c) =(a) * (b)	(d)	(e)	(f) = (d) * (e)	(g) = (c) * (f)	
Experimental unit #	RSP	Average total fish length	Water velocity	Tank dimensions width	depth	cross area	Water flow required	
	bl/s	cm	cm/s	cm	cm	cm^2	cm^3/s	L/min
Raceway 1	1	9.26	9.26	12.3	1.4	17.22	159.42	9.57
Raceway 2	1	9.04	9.04	12.3	1.3	15.99	144.56	8.67
Raceway 3	1	9.07	9.07	12.3	1.4	17.22	156.22	9.37
Raceway 4	1.5	8.74	13.11	9.3	1.4	13.02	170.68	10.24
Raceway 5	1.5	8.84	13.27	9.3	1.3	12.09	160.40	9.62
Raceway 6	1.5	8.65	12.97	9.3	1.3	12.09	156.81	9.41
Raceway 7	0.5	8.75	4.38	15.3	1.2	18.36	80.36	4.82
Raceway 8	0.5	9.52	4.76	15.3	1.2	18.36	87.39	5.24
Raceway 9	0.5	9.46	4.73	15.3	1.3	19.89	94.08	5.64

Table 4.6.- Flow required for each raceway at the beginning of Week 9 based on the average of total fish length within a given raceway and the targeted relative swimming velocity (RSP).

	(a)	(b)	(c) =(a) * (b)	(d)	(e)	(f) = (d) * (e)	(g) = (c) * (f)	
Experimental unit #	RSP	Average total fish length	Water velocity	Tank dimensions width	depth	cross area	Water flow required	
	bl/s	cm	cm/s	cm	cm	cm^2	cm^3/s	L/min
Raceway 1	1	10.27	10.27	12.3	1.4	17.22	176.83	10.61
Raceway 2	1	9.93	9.93	12.3	1.3	15.99	158.74	9.52
Raceway 3	1	9.94	9.94	12.3	1.4	17.22	171.13	10.27
Raceway 4	1.5	9.41	14.12	9.3	1.4	13.02	183.78	11.03
Raceway 5	1.5	9.62	14.43	9.3	1.3	12.09	174.41	10.46
Raceway 6	1.5	9.63	14.45	9.3	1.3	12.09	174.64	10.48
Raceway 7	0.5	9.65	4.82	15.3	1.2	18.36	88.57	5.31
Raceway 8	0.5	10.34	5.17	15.3	1.2	18.36	94.92	5.69
Raceway 9	0.5	10.48	5.24	15.3	1.3	19.89	104.21	6.25

Table 4.7.- Culture coverage area within each raceway at the beginning of Week 1.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	15	15	15	15
TSA/fish (cm^2)	8.43	9.17	9.18	8.75	9.66	8.52	9.14	9.62	8.93
PCA (%)	32	34	34	43	48	42	28	29	27

Table 4.8.- Culture coverage area within each raceway at the beginning of Week 3.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	15	15	15	15
TSA/fish (cm^2)	14.23	13.89	14.51	14.50	14.74	12.90	14.21	15.60	14.86
PCA (%)	53	52	54	72	73	64	43	47	45

Table 4.9.- Culture coverage area within each raceway at the beginning of Week 5.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	15	15	15	15
TSA/fish (cm^2)	21.58	20.98	21.25	20.82	20.73	18.56	20.47	22.91	22.01
PCA (%)	81	79	80	103	103	92	62	69	66

Table 4.10.- Culture coverage area within each raceway at the beginning of Week 7.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	15	15	15	15
TSA/fish (cm^2)	26.38	25.59	26.14	23.83	24.80	23.68	23.87	28.15	27.80
PCA (%)	99	96	98	118	123	115	72	85	84

Table 4.11.- Culture coverage area within each raceway at the beginning of Week 9.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	14	14	15	15
TSA/Fish (cm^2)	32.70	30.62	31.36	27.64	28.98	30.12	28.62	32.81	34.22
PCA (%)	95	89	91	106	111	103	62	77	80

Table 4.12.- Culture coverage area within each raceway at the end of Week 10.

Raceway	1	2	3	4	5	6	7	8	9
Fish/raceway	15	15	15	15	15	14	14	15	15
TSA/Fish (cm^2)	36.33	34.09	35.63	30.31	33.02	34.22	32.31	36.85	38.52
PCA (%)	105	99	103	116	127	111	70	86	90

Table 4.13.- Fish mass and morphometric data for California halibut raised at three water flow velocities for ten weeks. Values followed by a different letter on the same row are significantly different ($p < 0.05$).

	Water velocity (bl/s)					
	0.5		1.0		1.5	
	Average	SD*	Average	SD	Average	SD
W (g)						
Initial	1.60	0.11	1.49	0.07	1.51	0.20
Final	16.56a	0.41	14.66ab	1.26	12.69b	0.78
TL (cm)						
Initial	5.44	0.12	5.31	0.09	5.36	0.18
Final	11.01a	0.11	10.61ab	0.20	10.04b	0.18
TSA (cm^2)						
Initial	9.23	0.35	8.93	0.43	8.98	0.60
Final	37.69a	0.84	35.35ab	1.15	31.66b	1.36
BSA (cm^2)						
Initial	6.07	0.23	5.77	0.26	5.90	0.45
Final	25.30a	0.54	23.29ab	1.18	21.05b	0.87

* SD = standard deviation

Table 4.14 list fish growth and feed efficiency for the entire experimental period. A decrease in the growth and feed efficiency for fish reared at the highest (1.5 bl/s) in comparison to the lowest (0.5 bl/s) water velocities was observed. Significant differences ($p < 0.05$) were found among the three levels of water flow velocities tested for PWG, SGR, TSAG, BSAG, BG, and FE. There were no significant differences on CV among treatment groups (Table 4.14). California halibut reared at 0.5 bl/s grew more and had a better FE than those reared at 1.5 bl/s, but were not different from the ones reared at 1.0 bl/s.

4.4.3.- Analysis of growth every other week for ten weeks

Average masses (W) increased significantly ($p<0.05$) from 1.36-1.74 g at the start of the experiment to 11.90-16.96 g after 10 weeks for the three rearing velocities (Fig. 4.5). The difference in mean W among treatments increased over time and become significant ($p<0.05$) among swimming velocity groups on Week 10 (Fig. 4.5). At Week 10, W for fish reared at 0.5 bl/s was larger ($p < 0.05$) than for fish reared at 1.0 bl/s and at 1.5 bl/s (Fig. 4.5).

Coefficients of variation (CV) were highly variable over time and among treatments (Fig. 4.6). The largest overall average CV, but not significantly different, was observed for treatment 0.5 bl/s, while treatments 1.0 and 1.5 bl/s were similar (Fig. 4.6).

Table 4.14.- Fish growth and feed efficiency for California halibut raised at three water flow velocities for ten weeks. Values followed by a different letter on the same row are significantly different ($p < 0.05$).

	Water velocity (bl/s)					
	0.5		1.0		1.5	
	Average	SD	Average	SD	Average	SD
PWG (%)	909.04a	69.18	882.00a	98.45	702.33b	26.26
SGR (%/d)	3.98a	0.12	3.93a	0.17	3.59b	0.06
TSAG (%)	307.21a	24.10	296.98a	30.65	244.14b	2.18
BSAG (%)	314.16a	23.26	304.82a	35.14	248.00b	6.86
BG (g)	223.65a	7.21	197.43a	18.99	166.50b	9.48
FE (g biomass / g feed)	0.70a	0.03	0.70a	0.03	0.63b	0.01
CV (%)	29.89a	2.87	26.32a	3.63	25.41a	2.97

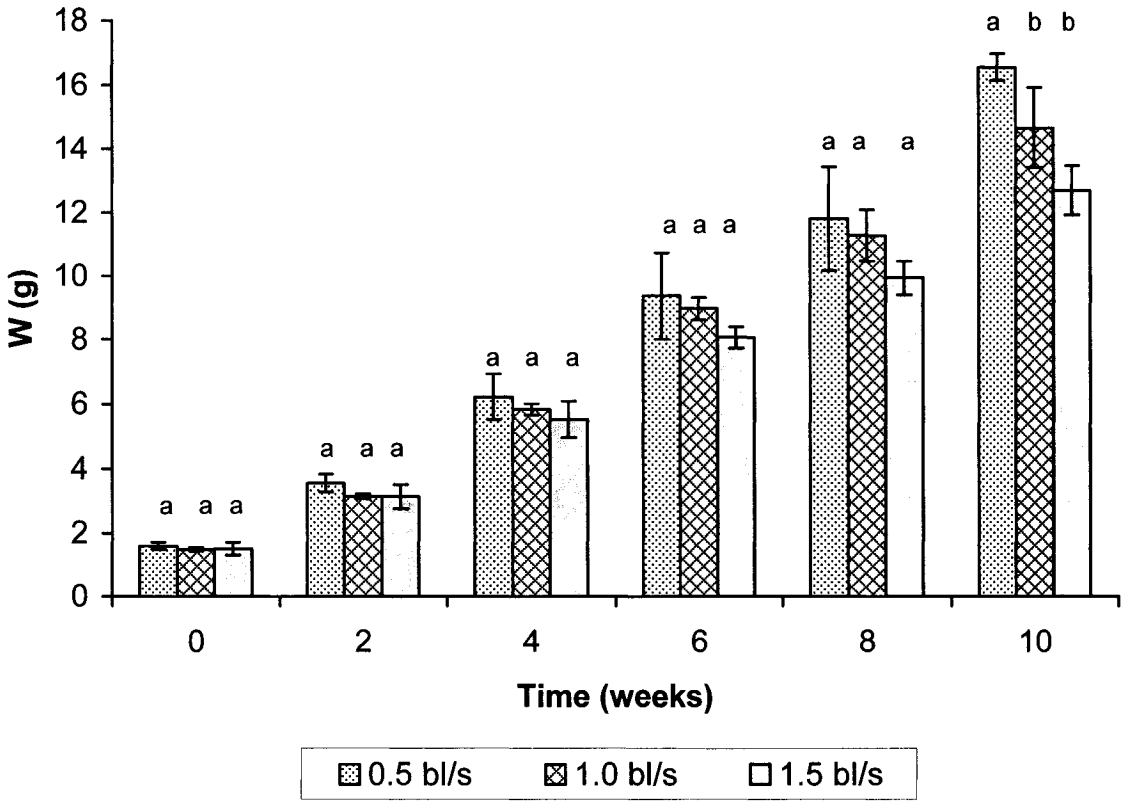

Figure 4.5.- Comparison of growth of California halibut juvenile during the 10 weeks under three water velocities. Columns having a different letter are significantly different ($p < 0.05$).

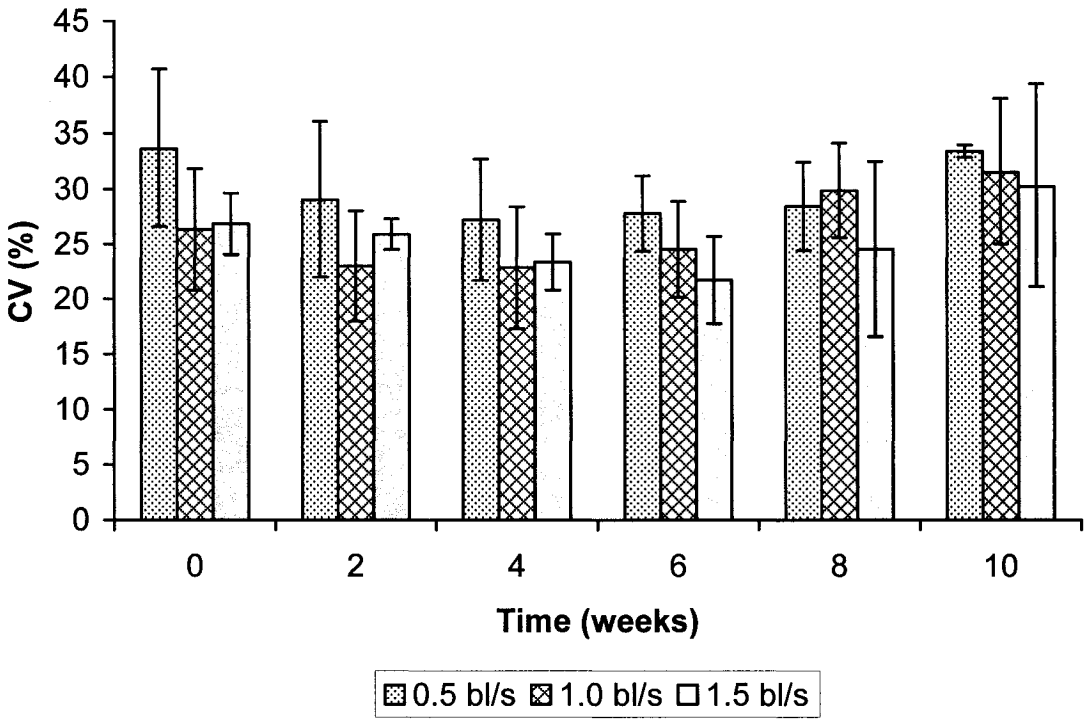

Figure 4.6.- Mean coefficient of variation of California halibut juveniles at the three rearing densities during a 10 week period. There were no significant differences among CV on any given week.

Mean specific growth rates (SGR) decreased from Week 2 to Week 8, and increased again for Week 10 (Fig. 4.7). The pattern of mean SGRs among the three relative swimming velocities remained similar during the study (Fig. 4.7). However, mean SGR of California halibut juveniles differed significantly ($p < 0.05$) among the treatments on Week 10 being the smallest for fish held at 1.5 bl/s (Fig. 4.7).

4.5.- DISCUSSION

4.5.1.- Water quality

California halibut studies on the effects of water quality parameters, such as temperature and salinity, on growth have been made by Innis (1980) and Madon (2002). Salinity and temperature were 30.7±0.9 g/L and 21.9±0.2 °C, respectively, which were within the recommended values given by Innis (1980) and Madon (2002) for culture of California halibut juveniles.

There have been no studies of California halibut regarding adequate levels of TAN, nitrite, and pH, and there is very little information for other flatfish species in the literature. Maximum levels up to 13 mg TAN/L have been reported as a safe concentration for the culture of turbot (Person-Le Ruyet, et al., 1997). In the case of NO_2-N, a recommended safe level for recirculating mariculture systems is 0.5 mg NO_2-N/L (Blancheton, 2000; Blanchard et al., 2003). Overall, values monitored in this research for TAN (0.01 to 0.8 mg/L), NO_2-N (0.19 to 1.52 mg/L), and pH (7.34 to 8.10) were within the recommended values with few exceptions during Week 8 of the experiment.

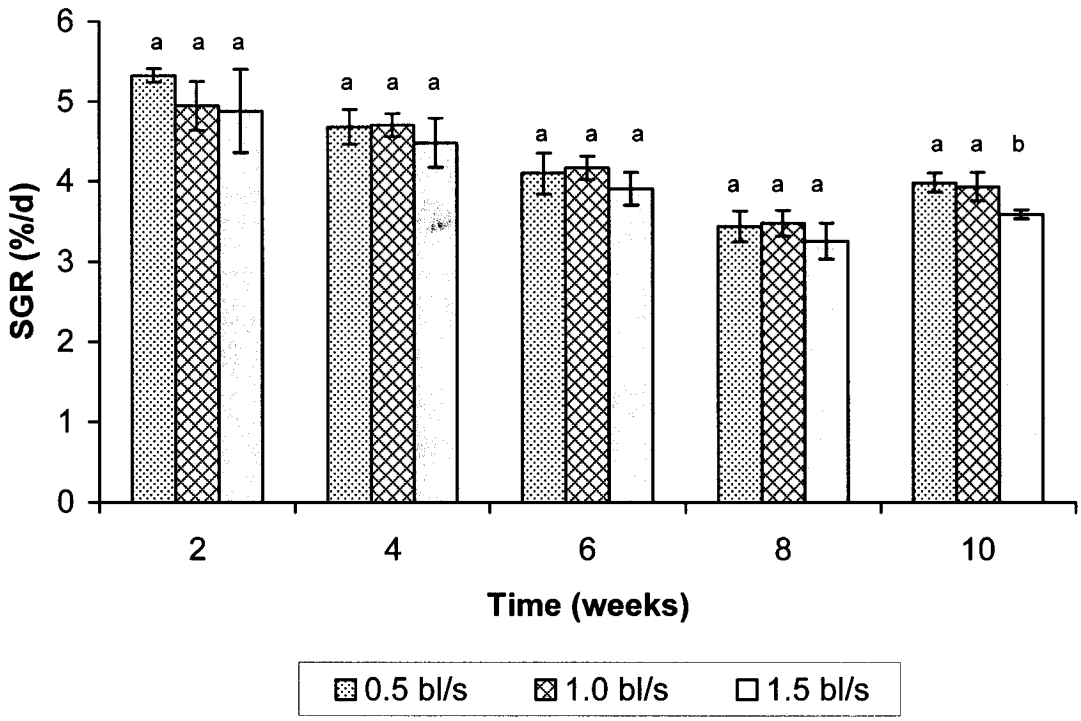

Figure 4.7.- Specific growth rate for California halibut juveniles reared at three velocities during ten weeks. Columns having a different letter are significantly different ($p < 0.05$).

Required dissolved oxygen levels, expressed as oxygen saturation, among flatfish are species-specific with minima ranging from 30 to 60 % of oxygen saturation, or 4 to 6 mg O_2/L depending on water temperature and salinity (Brown et al., 1984; van der Thillart et al., 1994; Petersen and Pihl, 1995; Tallqvist et al., 1999). Under the experimental conditions of the current research, the dissolved oxygen in the water entering the experimental tanks was 6.76±0.15 mg/L (~90% saturation). An oxygen consumption equation proposed by Brown et al. (1984) for turbot was utilized to estimate the oxygen consumption of California halibut reared in this research:

$$\ln O_2 = -0.2891 \ln W - 0.3345 \qquad \text{(Eq. 4.10)}$$

where O_2 = oxygen consumption under 16 °C (g O_2 / kg fish per h)

W = fish mean mass (g)

The worst rearing condition was for fish within the 0.5 bl/s treatment. Hence to estimate the effluent oxygen concentration a mass balance analysis (Timmons et al., 2001) was performed to the experimental tanks with 0.5 bl/s treatment assuming steady state conditions. Due to low biomass within the tanks and the high water exchange (> 4 volume changes/min), a negligible dissolved oxygen decrease of under 3% of influent concentration should be expected.

4.5.2.- Analysis of growth between weeks 0 and 10

Several studies have confirmed that sustained exercise leads to increase mass in salmonids (Davison and Goldspink, 1977; Totland et al, 1987; Christiansen and Jobling, 1990). In the present research, the rearing of California halibut juveniles at relative swimming velocities of 1.5 bl/s failed to promote growth. Juvenile California halibut grew best when reared at a relative swimming velocity of 0.5-1.0 bl/s. This agrees with the findings of Ogata and Oku (2000), who obtained similar results with hirame, and who suggested that the optimum water velocity for growth occurred at about 1.0 bl/s (9-16 cm TL fish). Bengtson and Alves (2002) found that summer flounder (124 g, 257 g, and 387 g), grew best when reared at ~0.5 bl/s in comparison to those grown at relative swimming velocities less than 0.3 bl/s or greater than ~1.3 bl/s.

Juvenile California halibut grown at a relative velocity of 1.5 bl/s showed a significant reduction in mean population mass gain (PWG), total surface (TSAG) and body surface (BSAG) gains, and specific growth rate (SGR), in comparison to the other treatments studied (Table 4.14). Consequently fish that were 162 dph reared at 1.5 bl/s were smaller than those which were grown at 0.5-1.0 bl/s.

It might be possible that a fish exposed to exercise will eat more to provide the energy required to sustain swimming activity. California halibut within all treatments were fed at the same ration, based on percentage of population mass, twice a day for 30 minutes. Studies with brook trout (Leon, 1986) have

shown that fish grow best at higher current velocities if fed to satiation. But feeding to satiation did not improve growth for red sea bream (Forster and Ogata, 1996) nor for hirame (Ogata and Oku, 2000). Furthermore, when feed rate was based on percentage of population mass, the medium and fast swimming red sea bream were unable to utilize the feed for growth as efficiently as were the slow swimming controls (Forster and Ogata, 1996). Similar results were found here, in which the lowest feed efficiency for California halibut occurred at 1.5 bl/s (Table 4.14).

Arnold and Weihs (1978) stated that, at high water velocities, the pressure on the dorsal side of a flatfish resting on the bottom of a tank will be less than the pressure on the ventral side; hence a lift force will be produced. If the lift force is greater than the fish negative buoyancy, the fish will be lifted unless it produces another force to maintain its attachment to the bottom. Flatfish species are adapted to counteract hydrodynamic lift and maintain station on the bottom (Arnold, 1969). Plaice was found to lie on the bottom and remain motionless up to about 1 bl/s before slipping or displacement occurred (Arnold and Weihs, 1978). Arnold (1969) observed that water velocities that produced a relative velocity of ~1.5 bl/s increased the beating of the posterior fin in plaice which began to swim when the relative velocity reached ~2.0 bl/s. Similarly, Ogata and Oku (2000) described a constant waving of the posterior fin in hirame at 1.0 and 2.1 bl/s. Waving of posterior fins was also observed in this study for California halibut maintaned at 1.0 and 1.5 bl/s. This beating of the posterior fin was

suggested as a mechanism to maintain position on the bottom against the water current (Arnold, 1969; Arnold and Weihs, 1978; Ogata and Oku, 2000). Ogata and Oku (2000) explained, for hirame, that the required metabolic energy to maintain position exceeded the physiological ability of the fish to deposit lipids in fin muscles, when exposed to 2.1 bl/s, which lead to slow growth and a poor feed efficiency. It seems, then, that in this research the juvenile California halibut exposed to the higher water velocity could have used more energy for metabolic activity instead of somatic growth, which would explain the lower biomass gain (BG) observed at 1.5 bl/s. However, further studies of this matter are required.

Another possible cause of depressed growth in flatfish is the amount of surface area available for culture. There are no data currently available for juvenile California halibut that can suggest a density at which growth rate will not be depressed. However, for other flatfish, it has been reported that culture density below 150% of coverage area does not negatively affect the growth (King et al., 1998; Irwin et al., 1999). In this research, culture densities were between 28 and 123% PCA, hence lower than 150% PCA, and therefore unlikely to have affected fish growth. However, further studies of this matter are required.

Loss of biomass and a reduction in specific growth rates have been reported for flatfish species when they have been grown in still water or at relative swimming velocities less than 0.3 bl/s (Bengtson and Alves, 2002; Ogata and Oku, 2000). So, it appears as though there is a relative swimming velocity at which biomass

gain can be maximized in flatfish. Based on the results of this research, juvenile California halibut can be raised at relative swimming velocities as low as 0.5 bl/s without affecting their growth performance. However, further studies are required to determine growth performance at relative swimming velocities under 0.5 bl/s for California halibut.

4.5.3.- Analysis of growth every other week for ten weeks

Fish farmers would like their fish to grow rapidly, make efficient use of feed, and have a uniform size at harvest. In this research the largest mean fish biomass along the ten experimental weeks was observed for fish under a relative swimming velocity of 0.5 bl/s. Additionally, SGR were similar and greater for fish within the 0.5 and 1.0 bl/s treatments, than for those at 1.5 bl/s. In flatfish, variation in individual growth rates is reported to be related more to social interactions than to feed availability (Irwin et al, 1999). Hence, having fish with uniform size will require manipulation of biotic factors (e.g., feeding frequency, ration size and behavioral interactions) (Jobling et al., 1993).

The manipulation of biotic factors could enhance fish growth rates while decreasing size variation (Jobling et al., 1993; Jobling, 1994; Irwin et al., 1999). One important biotic factor that influences the growth of fish is the development of hierarchies, mediated by intra-specific competition for feed (Irwin et al., 1999). Hierarchies, which may lead to an increase of aggressive interactions, can be stimulated at lower water velocities (East and Magnan, 1987; Christiansen and

Jobling, 1990; Jobling et al., 1993). In this research the largest CV, although not significantly different, was observed for fish within treatments with a relative swimming velocity of 0.5 bl/s (Fig. 4.6). Both 1.0 and 1.5 bl/s had a low CV along the ten weeks, which means that hierarchy was not likely to have a significant effect on growth rates in these groups in comparison to those groups stocked at 0.5 bl/s.

Hierarchies at high stocking densities leading to the suppression of growth have been reported for flatfish (King et al., 1998; Irwin et al, 1999; Fairchild and Howell, 2001). In this research, stocking densities were less than 150% PCA, which has been suggested as the maximum PCA to maximize fish growth for flatfish and at which hierarchy formation has not been fully developed (King et al., 1998; Irwin et al, 1999; Fairchild and Howell, 2001). Because it has been suggested that high stocking density *per se* may lead to improved growth through influences on social behavior (Jobling et al., 1993; Irwin et al, 1999), it is advisable that further studies of stocking density and relative swimming velocity be done for California halibut.

There are other factors not explored in this study that could affect the swimming activity in fish, such as fish size, water temperature, sex, light, food, and training (Hammer, 1995). Defining a range of water velocities which support California halibut growth under all conditions would be difficult, as it is likely that performance would vary according to life stage and husbandry practices. To

develop management strategies which optimize the production of California halibut in flowing water, future studies are needed which identify life-stage-dependent culture requirements over a range of water velocities.

4.6.- CONCLUSIONS

The most relevant conclusions from the present chapter are:

a) There is no promotion of growth on California halibut juveniles reared for ten weeks at velocities of up to 1.5 bl/s. Hence the statistical null hypothesis was not rejected under the current experimental conditions at a significance level $\alpha = 0.05$.

b) California halibut juveniles grew faster and made more efficient use of the feed provided at relative swimming velocities of 0.5 and 1.0 bl/s than at 1.5 bl/s.

c) A larger coefficient of variation was found for fish in the 0.5 bl/s than in 1.0 bl/s and 1.5 bl/s treatment, indicating higher interactions among fish and less uniformity in size.

d) California halibut juveniles can be raised between 0.5 and 1.5 bl/s without affecting their survival, but in terms of growth it is advisable not to operate over 1.0 bl/s.

4.7.- FURTHER RESEARCH

As there are many factors that affect the swimming velocity of a fish, it is suggested to study California halibut for relationships between optimum relative swimming velocity and sex, size, feed and temperature. Some studies involving temperature have been done to detect preferences (Innis, 1980) or growth differences (Gadomski and Caddell, 1991; Madon, 2002), but not in relation to water flow velocity.

The growth of California halibut as affected by swimming velocity and stocking density interactions should also be studied. Based on the literature reviewed, it was understood than relative swimming velocity and stocking density can affect flatfish growth.

CHAPTER V

EFFECT OF FISH STOCKING DENSITY ON GROWTH OF CALIFORNIA HALIBUT JUVENILES

5.1.- INTRODUCTION

Rapid fish growth under culture requires that the fish be provided with correctly formulated feeds and a good rearing environment. There are also biotic factors that can influence feeding responses and growth performance. Among the biotic factors affecting growth performance are fish size (Innis, 1980, King et al., 1998; Pennel and McLean, 1996), fish behavior (Sunde et al., 1998; Irwin et al., 1999), and sustained exercise (Jobling, 1994; Ogata and Oku, 2000).

The importance of stocking density on fish growth has been reported for several finfish species, such us rainbow trout (Trzebiatowski et al., 1981), Nile tilapia (Carro-Anzalota and McGuinty, 1986), hamoor (*Epinephelus tauvina*) (Abdullah et al., 1987), Arctic charr (*Salvelinus alpinus*) (Christiansen et al., 1992), African catfish (*Clarias gariepinus*) (Kaiser et al., 1995), gilthead sea bream (*Sparus aurata*) (Canario et al., 1998), and lake sturgeon (*Acipenser fulvescens*) (Fajfer, et al., 1999). There are a few studies on the effect of stocking densities on growth of flatfish: turbot (Martinez-Tapia and Fernandez-Pato, 1991; Irwin et al.,

1999; Irwin et al., 2003), hirame (Jeon et al., 1993), Atlantic halibut (Bjornsson, 1994), and summer flounder (King et al., 1998).

Both positive and negative relationships between stocking density and growth have been reported, and the pattern of this interaction appears to be species specific. A number of parameters directly related to stocking density and growth rate performance must be considered to optimize fish production systems. These include water quality, water velocity, nutrition, social behavior, and type and size of the rearing tank (Christiansen et al., 1992; Malison and Held, 1992; Jobling et al., 1993; Jobling, 1994; Canario et al., 1998).

High stocking density can lead to a deterioration of the water quality. Water quality (e.g. dissolved oxygen, ammonia nitrogen) has been found to affect growth rate of many fish species. Required dissolved oxygen levels among flatfish are species-specific with minima ranging from 4 to 6 mg O_2/L depending on water temperature and salinity (Brown et al., 1984; van der Thillart et al., 1994; Petersen and Pihl, 1995; Tallqvist et al., 1999). Maximum levels up to 13 mg TAN/L have been reported as a safe concentration for the culture of turbot (Person-Le Ruyet, et al., 1997). High fish stocking densities generally require high water flow rates into the tanks to supply the oxygen and to carry out the metabolic byproducts (Elliot, 1969; Brown et al., 1984; Bergheim et al., 1993).

A factor closely related to flow rate is current velocity, which can significantly affect fish performance in culture tanks. In Chapter V it was concluded that growth rate of Califonia halibut juveniles was not reduced if the fish were reared at less than 1.0 bl/s.

Social behavior is considered to be among the major factors associated with the stocking density and growth rate performance of a particular species (Jobling, 1994; Ross et al., 1995). It is generally assumed that social interactions through competition for feed and/or space can negatively affect fish growth. An increase in swimming activity with increases in stocking density has been reported for Atlantic halibut, which gave a lower feed efficiency at high stocking density compared to intermediate stocking density; hence more of the feed was used for metabolism (Bjornsson, 1994). It has also been reported that competition between individuals for a limited feed supply resulted in non-uniform feeding and this ultimately gives rise to differential growth. Martinez-Tapia and Fernandez-Pato (1991) reported increased aggressiveness and competition in turbot under conditions of feed scarcity. Lower feed consumption at high densities could be the result of lower efficiency in the search of feed; fish having more difficulty moving and reaching the feed at higher stocking densities (Refstie and Kittelsen, 1976). Carter et al. (1996) demonstrated a positive relationship between feed consumption and growth in greenback flounder, and suggested that growth dispensation was due to differential rates of feed consumption. On the other

hand, a direct correlation between stocking density and growth rate has been reported for hirame (Honda, 1988; Jeon et al., 1993).

Fish rearing stocking densities (biomass per unit of rearing space) and the design of the rearing container have been extensively examined to promote efficiency in fish culture (Ross et al., 1995; Ewing et al., 1998; Lambert and Dutil, 2001). Rearing densities define the physical space required for optimum growth and health of the fish. In addition it has been reported that swimming behavior, fish interactions, feed dispersion, water quality, water flow, and water velocity patterns may differ for fish reared at higher stocking densities in small and large tanks (Cripps and Poxton, 1992; Ross et al., 1995) . Flatfish species are bottom dwelling fish and to enhance growth and survival it has been recommended to provide high substrate to water area ratio rather than a high water volume (Cripps and Poxton, 1992; Oiestad, 1999). It has been shown for a bottom dwelling fish that bottom surface area was an important design criteria to achieve higher stocking densities (Kaiser et al., 1995).

Stocking density for flatfish may be expressed either as biomass per unit of bottom area or as a percentage of coverage area (PCA = ratio of total fish ventral area to total tank bottom area). Commercial land-based facilities usually stock their fish at densities as high as 40 kg/m^2 for hirame (Honda, 1998), 70 kg/m^2 for sole (Liewes, 1984), and up to 120 kg/m^2 for turbot (Person Le-Ruyet et al, 1983). However, the normal operational range for flatfish species varies from 15

to 50 kg/m^2 depending on rearing conditions (Jones, 1981; Person Le-Ruyet et al., 1983; Liewes, 1984; Silva and Velez, 1998; Martinez-Tapia and Fernandez-Pato, 1991; Mallekh et al., 1998; Kikuchi, 2000). Bjornsson (1994) suggested that for a 2 kg Atlantic halibut the optimal stocking density is between 25 and 50 kg/m^2 and for 10 kg Atlantic halibut between 50 and 100 kg/m^2. The suggested safe stocking density for flatfish is between 100 and 200% coverage area (Jeon et al., 1993; Bjornsson, 1994; King et al, 1998).

California halibut is a gregarious species in the wild and when stocked in tanks, therefore high stocking densities might be possible for a commercial aquaculture system (James Rounds, California Halibut Hatchery Program. Personal communication, 2001). To date, no studies have been published that examine stocking density for California halibut. The ability to raise California halibut at a relatively high density, thus maximizing culture area usage, is of particular importance for future commercial operations. Consequently, this research was planned to estimate the effect that stocking density may have on the growth rate of California halibut juveniles.

5.2.- EXPERIMENTAL DESIGN

A study was carried out to test three stocking densities on fish performance. The study evaluated three stocking densities, 100, 200, and 300 % PCA. Stocking densities were run in triplicate in small tanks (experimental tanks) attached to the

California Halibut Recirculating Hatchery (Appendix A.1). Fish morphometrics (total length, TL; total surface area, TSA; body surface area, BSA) were determined at the beginning of the experiment and at Week 8 by image analysis (Appendix A.2). Fish average mass (W) was measured at the beginning of the experiment and every other week thereafter. The stocking density experiment lasted ten weeks.

Three of the raceways within the California Halibut Recirculating Hatchery were stocked at 100, 200, 300 % PCA as were the experimental tanks. The purpose was to validate the use of the experimental tanks by comparing the fish population average mass (W) from raceways and experimental tanks. Stocking densities were not replicated for the raceways. After Week 6, the raceways stocked at 200 and 300% PCA started to have water quality problems due to limitations in flow capacity and the comparison was terminated.

5.2.1.- Fish stock

For this study 171 dph weaned California halibut grown at the California Halibut Recirculating Hatchery (Appendix A.1) were used. The juveniles (11.6 g wet mass) were reared in raceways on a recirculating seawater system with a constant temperature of 22 ± 1 °C and salinity of 30 g/L (Gadomski and Caddell, 1991; Madon, 2002). The fish were fed daily at ~1.2 % body mass with commercial dry pelleted feeds (EWOS™ Alpha #2; EWOS™ Pacific #3). Light

was provided by overhead fluorescent tubes on a 16 L: 8 D (L=light; D=dark) photoperiod (Boeuf and Le Bail, 1999; Klokseth and Oiestad, 1999a).

5.2.2.- Fish ventral surface area estimation

Fish density was measured as percentage of coverage area (PCA) of tank bottom. Fish of similar total length were selected to stock the experimental tanks and their ventral surface area was estimated by image analysis (Appendix A.2). Total length, total surface area (fish body including all fins), and body surface area (fish body excluding fins) were determined. Each measurement was paired with the respective fish wet mass. Image analysis was performed at the beginning of the experiment and again at the beginning of Week 8.

5.2.3.- Experimental tanks and raceways

Rectangular tanks with total internal length of 60 cm were used for the stocking density experiment (Fig. 5.1). Three tank widths were used to achieve the desired stocking densities: 9.3 cm, 12.3 cm, and 15.3 cm (Table 5.1). Screens (5 mm aperture) were placed at both ends of the tank to hold the fish within the experimental area. The water depth in each tank was maintained at ~6 cm by a weir located at the end of the tank (second weir in Fig. 5.1). Another weir, the first weir, was placed with the effluent screen (Fig. 5.1). A quiescent zone was created between the weirs, allowing for observation of the amount of feed uneaten by the fish. Tanks were flushed every day in the morning and before feeding by removing the second weir for a few seconds. The function of the first weir was to keep a constant water level of ~2 cm during the 'flushing' process.

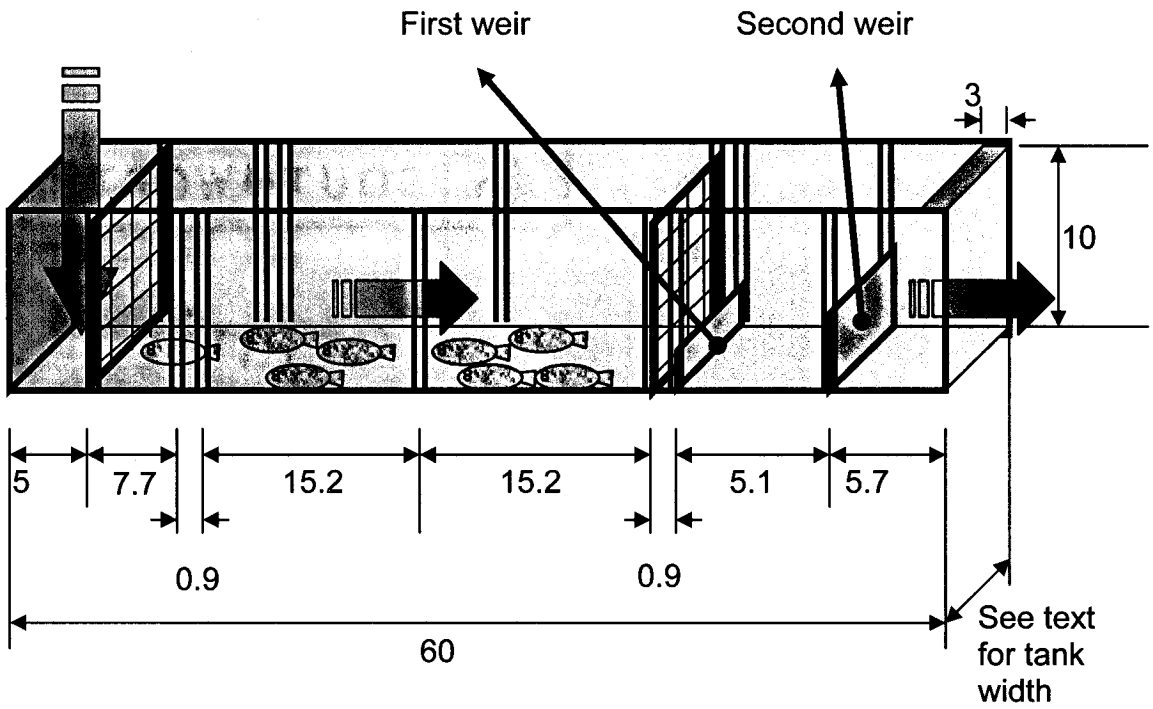

Figure 5.1. Schematic diagram of a tank used for the density experiment. All measurements are shown in cm. Each slot to introduce nets or weirs is 0.6 cm wide (measurement not shown). Figure is not to scale. Large arrow indicates the direction of the water flow. Tanks width were 9.3, 12.3, and 15.3 cm. Water depth is not shown but it was ~6.0 cm. Tanks were built from dark grey acrylic glass, 0.5 cm thick.

Table 5.1.- Dimensions for tanks. Tanks 1 to 3 were stocked at 200% PCA, 4 to 6 at 300% PCA, and 7 to 9 at 100% PCA. Fish were stocked in a 41 cm long tank section. Water depth was maintained at ~6 cm.

Tank	1 to 3	4 to 6	7 to 9
Width (cm)	12.3	9.3	15.3
Surface (cm^2)	504.3	381.3	627.3

The raceways used were 2.41 m in length, 0.28 m in width, and 0.22 m in height (Fig. 5.2). A 19 cm quiescent zone was located at the effluent section. The tank surface area available for fish rearing, excluding the quiescent zone was ~6105 cm^2. The water level was maintained between 3.5 and 4.5 cm by an external standpipe. Cylindrical nets (5 mm aperture) were placed at the influent (Fig. 5.3) and effluent (Fig. 5.4) for the purpose of preventing fish from escaping and to provide a frame support for a dissolved oxygen sensor.

5.2.4.- Experiment 1: Morphometrics and growth in tanks. Culture protocol

California halibut juveniles to be stocked within the tanks were obtained from a common population (n = 1840) with an average of 11.6 g wet mass (W), which was obtained by counting and weighing the fish as a group. An expression that quantifies the relationship between W and TSA was used to determine the fish size to be stocked within the tanks. The expression was determined from the data presented in Chapter IV (Appendix A.2; Fig. A2.4) and is given as:

$$TSA\ (cm^2) = 7.08 \times W\ (g)^{0.6056},\ r^2 = 0.99 \qquad \text{(Eq. 5.1)}$$

The number of fish to be stocked within the tanks and raceways was estimated based on a TSA of 31.24 cm^2/fish, the culture area available (Table 5.1), and the respective stocking density (100, 200, or 300%PCA). Three stocking densities of 100, 200 and 300% PCA were established by stocking 20, 32, and 36 individuals

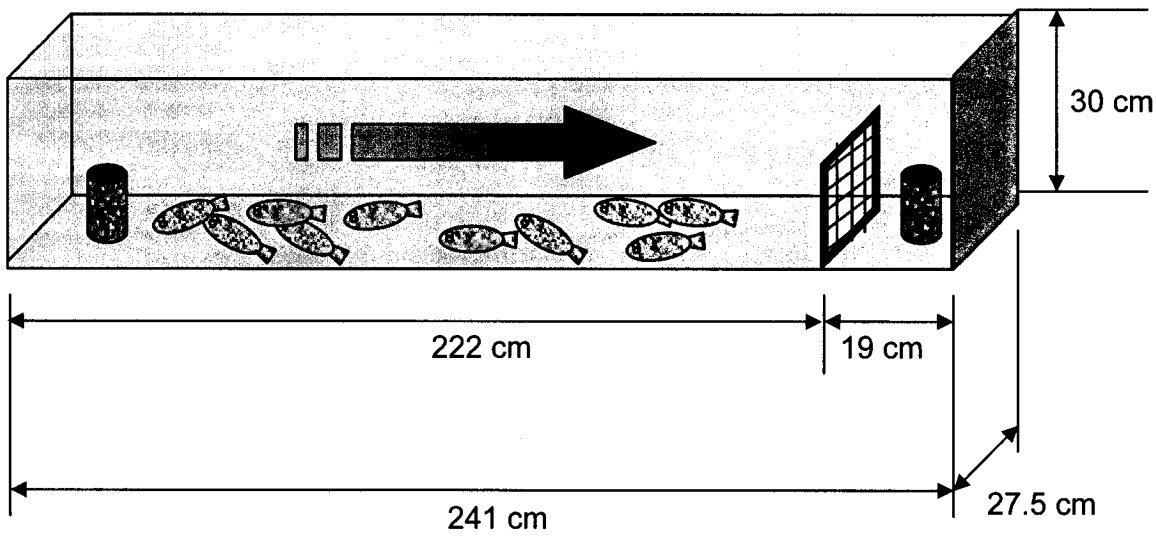

Figure 5.2. Schematic diagram of a raceway. Water flow was from left to right (arrow). Water depth was between 3.5 and 4.5 cm. Figure is not to scale.

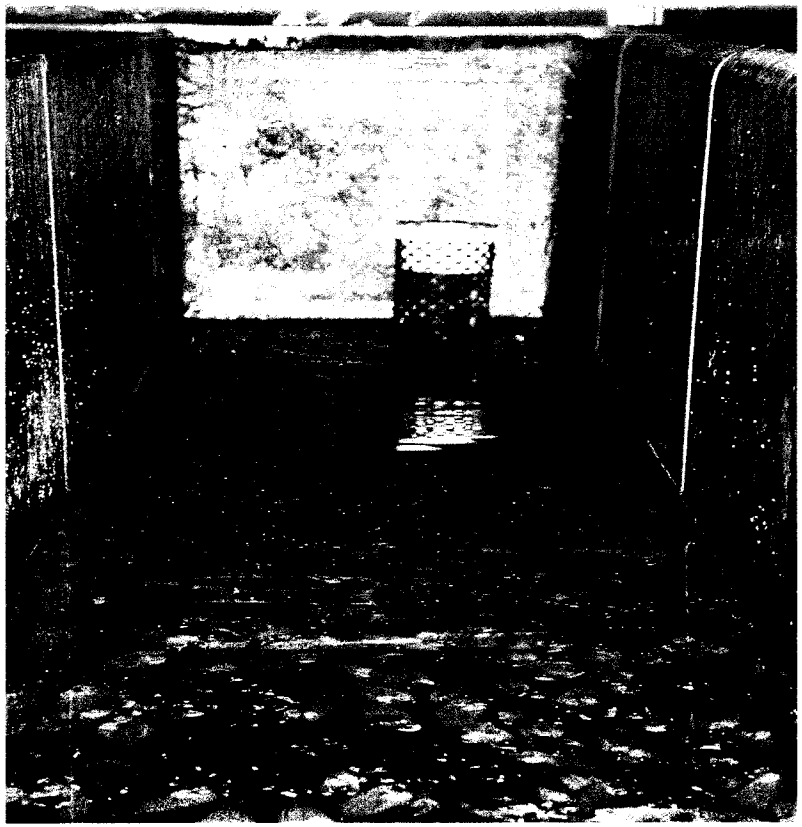

Figure 5.3.- View of influent zone in Raceway 1, stocked at 300% coverage area. Cylindrical screen is placed on the influent pipe section.

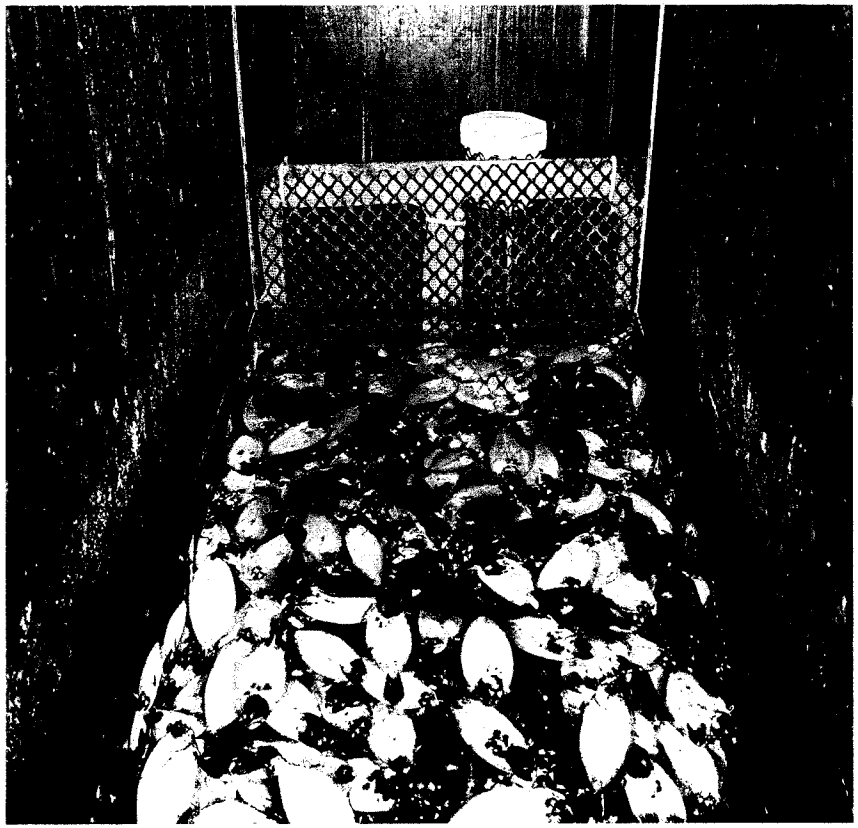

Figure 5.4.- View of effluent zone in Raceway 1. A screen was used to create a quiescent zone and a cylindrical screen was used as a frame to support a dissolved oxygen probe (probe not installed for this picture).

respectively into each of the three replicate tanks per treatment (Table 5.1). Fish required for the study were pulled out from the fish population based on their total length (TL). An expression that quantifies the relationship between W and TL was used to determine the required fish TL. The expression was determined from the data presented in Chapter IV (Appendix A.2; Fig. A2.6) and is given as:

$$TL\ (cm) = 4.79 \times W\ (g)^{0.302},\ r^2 = 0.99 \qquad \text{(Eq. 5.2)}$$

And the target TL for W of 11.6 g was 10.1 cm.

The fish were fed commercial dry pelleted feeds (EWOS™ 1 and 2 mm) by automatic carrousel feeders (Lifegard® model AF2 from Aquatic Eco-Systems Inc) during the light hours (0900 to 2200 h) 7 d/wk for 10 wk. Individual fish biomass was measured at stocking time and then every other week to adjust the amount of feed, maintaining a ration of 1.2 % body mass per day. Tanks were flushed every morning before feeding to remove any uneaten pellets and feces by decreasing the water level from 6 to 2 cm. Fish mortalities were replaced during sampling time (every other week) with a fish that matched the average population morphological data for the respective replicate. The flow rates were changed every other week to achieve water velocities similar to those initially estimated for the raceways (see Section 5.2.5). Relative swimming velocity was estimated using the average total length of the fish stocked within a tank.

5.2.5.- Experiment 2: Fish growth in raceways and tanks. Culture protocol

This experiment was set up to compare growth between fish stocked within the raceways and the fish stocked within the tanks for six weeks. California halibut juveniles were taken randomly from a population of 1580 fish with estimated W of 11.6 g and an estimated TSA of 31.24 cm^2/fish (Eq. 5.1). The fish biomass to be stocked within the raceways was estimated based on the calculated 31.24 cm^2/fish, the tank culture area available (6105 cm^2), the respective stocking density (100, 200, or 300%PCA), and the estimated population W of 11.6 g/fish. Therefore it was estimated that about 2.3, 4.5, and 6.8 kg of fish should be stocked in the raceways to achieve the three density treatments of 100%, 200%, and 300% PCA, respectively. The exact number and biomass of fish stocked within the raceways and initial rearing conditions are shown in Table 5.2.

Water flow rates within the raceways were adjusted to have ~5 mg/L of dissolved oxygen at the effluent, which corresponded for 9, 12, and 15 L/min for the 100%, 200%, and 300% PCA treatment, respectively (Table 5.2). A maximum flow of 15 L/min per raceway could be achieved in the recirculating system; hence the raceway stocked at 300% PCA had the maximum flow allowed by the recirculating system (Table 5.2).

Raceways were cleaned daily in the morning before feeding by manually scraping the tank bottom, from influent to effluent section, to remove biofouling and dead fish. Mortalities were removed and counted daily from the raceways,

Table 5.2.- Culture conditions at time of stocking for raceways stocked at 100%, 200%, and 300% PCA.

PCA	Biomass	Fish	W	TL	Flow	Velocity	
%	g	#	g	cm	L/min	cm/s	bl/s
100	2404	214	11.23	10.0	9	1.1	0.09
200	4579	392	11.68	10.1	12	1.4	0.13
300	6795	616	11.03	9.9	15	1.8	0.16

and they were not replaced. Fish biomass was recorded every other week to adjust the amount of feed at a ration of 1.2 % body mass per day. The fish were fed commercial dry pelleted feeds (EWOS™ 1 and 2 mm) by automatic belt feeders (model BFS12A from Aquatic Eco-Systems, Inc.) during the light hours (0900 to 2200 h) 7 d/wk for 6 wk .

5.2.6.- Water quality measurements

Dissolved oxygen (DO) concentrations in the effluents from the tanks were estimated as in Chapter IV. And in the case of the raceways, average oxygen concentrations were recorded every 10 min with dissolved oxygen sensors (Sensorex model DO6000) located at their effluent and within the head tank. Temperature was monitored with a copper-constantan thermocouple (Omega®) placed within the reservoir. Data from oxygen sensors and thermocouples were recorded with a micrologger (Model 21X, Campbell Scientific, Inc.). Data recorded with the micrologger were uploaded to a PC computer using PC208 W 3.3 datalogger support communication software (Campbell Scientific Inc.). Data were uploaded every week and stored in a spreadsheet (Microsoft Excel 2002 SP-2). Other water quality parameters were measured as described in Appendix A.1.

5.2.7.- Fish sampling

All fish were sampled every other week to determine biomass so that feeding rations could be adjusted. Individual morphological data (total body length, TL in

cm; total surface area, TSA in cm^2; body surface area, BSA in cm^2) were gathered only for fish within the tanks (Appendix A.2) at the initial stocking time and at Week 8. Fish were unfed 24 h before sampling. Feeding was resumed the day after sampling. Culture density as a percentage of coverage area (PCA, %), culture density as biomass of fish per area (BFA, kg/m^2), coefficient of variation (CV, %), growth as percentage of mass gain (PWG, %), specific growth rate (SGR, %), biomass gain (BG, g), and feeding efficiency (FE, g/g) were estimated as in Chapter IV.

5.2.8.- Experimental design and statistical analysis

The statistical analysis to be performed in the present research is presented below for the fish reared in the tanks and for comparing fish performance between raceways and tanks.

5.2.8.1.- Experiment 1. Morphometrics and growth in tanks

The treatment factor called stocking density was considered as the independent variable at three fixed predetermined levels (fixed effect) of 100, 200, and 300% PCA with three replicates per treatment. The response variables analyzed were W, TL, TSA, BSA, SGR, PWG, TSAG, BSAG, BG, FE and CV. Response variables were analyzed for significant differences among treatments, using a one-way complete model analysis of variance (Dean and Voss, 1999) with SAS statistical software (release 8.02 Level 02M0). The stated null hypothesis (H_o) was that there is no difference in fish response between stocking density levels,

and the corresponding alternative hypothesis (H_i) was that at least one level is different. Significances were analyzed using Duncan's multiple range test and Tukey's studentized range test at a significance of 0.05 (Dean and Voss, 1999).

5.2.8.2.- Experiment 2. Fish growth in raceways and tanks

Experiment 2 started with three raceways stocked with densities similar to those of the tanks. The purpose was to validate the use of small vessels by comparing growth data from raceways and tanks. Raceways were not replicated, and therefore a single datum was used for analysis purposes. This experiment was terminated at Week 6 due to oxygen problems within raceways stocked at 200 and 300% PCA. The experiment considered two fixed factors, vessel type and stocking density. The vessel type factor had two levels, tank and raceway. The stocking density factor had three levels: 100, 200, and 300% PCA. The response variable W was analyzed for significant differences among treatments, using a two-way complete model ANOVA with negligible interaction (Dean and Voss, 1999) using SAS statistical software. There were two null stated hypotheses: (H_o) that there is no difference in fish growth between stocking density levels and (H'_o) there is no differences in fish growth between types of culture vessel used. The corresponding alternative hypotheses were (H_i) and (H'_i), where at least one level is different. The significance level of the ANOVA test was 0.01 due to the absence of replicates for the raceways, and to avoid making a Type I error (rejecting H_o when it is in fact true) (Dean and Voss, 1999). Significances were analyzed using Duncan's multiple range test and Tukey's studentized range test at a significance of 0.01 (Dean and Voss, 1999).

5.3.- RESULTS.

Raceways were stocked with 2404, 4579, and 6795 g of fish to achieve the three density treatments of 100%, 200%, and 300% PCA, respectively (Table 5.2). Water velocity in the tanks stocked at 100% PCA was initially adjusted to match the corresponding swimming velocity calculated for the 100% PCA raceway (Table 5.3). As a result, tanks stocked at 100% PCA had an initial loading rate of 21.3±0.5 L/min kg. Water quality differences among tanks can be minimized by having the same loading rate. Hence, the relative swimming velocity for the remaining tanks was set at the beginning of the experiment such that the loading rates were equal to those of the tanks stocked at 100% PCA. Thereafter, the tank flow rates were adjusted every other week to keep the initial relative swimming velocity (bl/s) constant over time (Table 5.3). Water velocities within the raceways and tanks induced relative swimming velocities below 1 bl/s.

5.3.1.- Water quality

Global average for salinity during the experiments was 30.1±1.3 g/L, and for temperature was 22.4±0.7 °C (Fig. 5.5). Temperature increased on February 21 and remained at 24.1±1.0 °C until March 18 due to a chiller failure. From March 19 to March 31 temperature was lowered to 19.6±1.2 °C using a new chiller. During the higher temperature period fish behaved normally, but TAN concentration increased from less than 1.0 mg/L to 5.5 mg/L. Nitrite nitrogen

Table 5.3.- Relative swimming velocity within the experimental tanks over time.

PCA	Relative swimming velocity (bl/s)						Average	SD*
	Week							
%	0	2	4	6	8	10	bl/s	
100	0.09	0.08	0.08	0.08	0.08	0.08	0.08	5.3E-03
200	0.18	0.17	0.17	0.16	0.16	0.16	0.16	1.2E-02
300	0.27	0.26	0.25	0.24	0.24	0.24	0.25	1.3E-02

*SD = standard deviation.

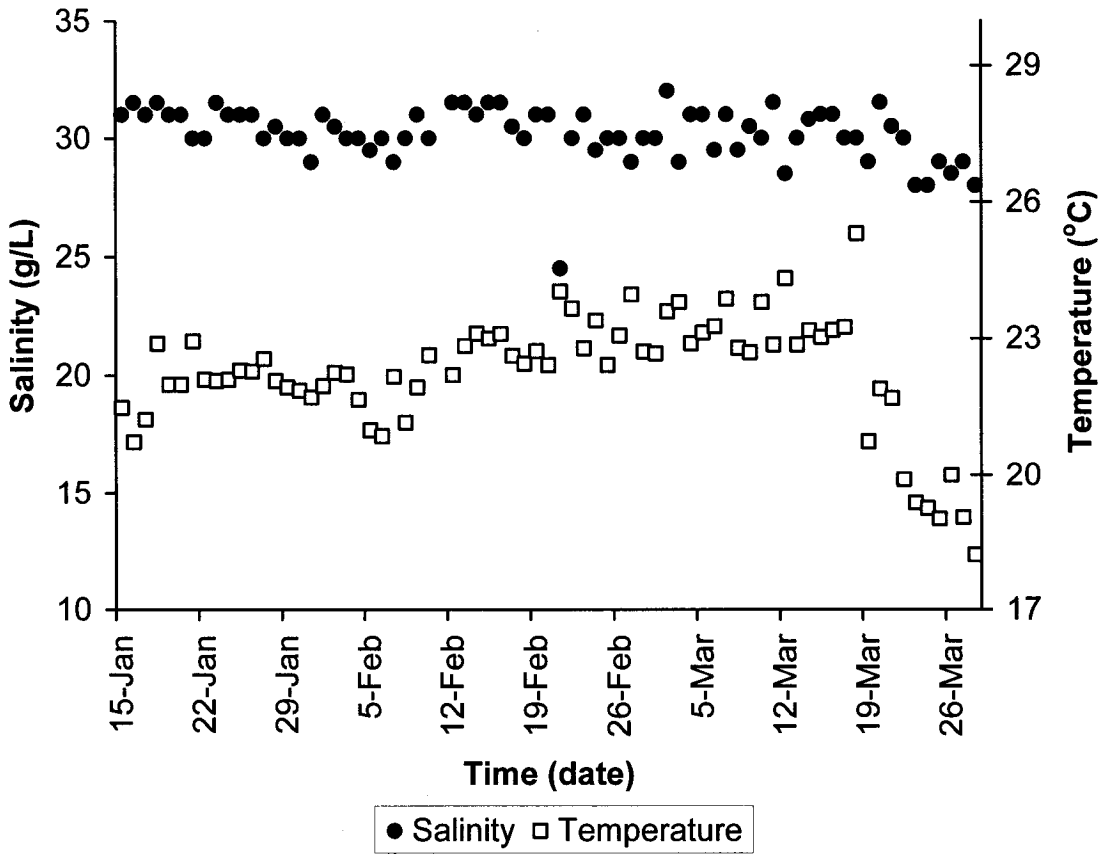

Figure 5.5.- Salinity and temperature within the seawater recirculating system during the 10 week experiment.

concentration varied between 0.2 and 0.9 mg/L. Water pH remained unchanged over time, having an average of 7.3±0.2. Alkalinity varied between 2 and 3 meq/L and had an average value of 2.4 ± 0.3 meq / L.

Dissolved oxygen in the head tank was greater than 95% of saturation. The lowest dissolved oxygen in the influent water to the experimental tanks was 6.38±0.09 mg/L. Effluent dissolved oxygen concentrations from the raceways reached 4.2±0.3, 4.4±0.4, and 4.9±0.4 mg O_2/L for 300% PCA, 200% PCA, and 100% PCA, respectively, during the sixth week, when the raceway experiment was terminated.

5.3.2.- Experiment 1. Morphometrics and growth in tanks

A total of five fish were replaced in replicates of the 300% PCA treatment, two of which died in the tanks and the other three jumped out of the tanks. Five fish were replaced in replicates of the 200% PCA treatment, one of which died in the tank and the other jumped out of the culture tank. No fish had to be replaced in replicates of the 100% PCA treatment.

Fish in the 300% PCA treatment tended to attach themselves to the wall of the tanks (Fig. 5.6). A total of three fish with damaged tails were found in the 300% PCA treatment tanks from Weeks 2 to 10. No injured fish were seen in any of the replicates of the 100% and 200% PCA treatments.

Figure 5.6.- General view of 300% coverage area at Week 10. Note fish attached to the tank walls (se arrows). Only two replicates are shown.

The water temperature rose to 24.1 ± 1.0 °C at the beginning of Week 7 and until the end of Week 9. This increase in temperature seemed not to affect fish behavior in terms of activity or hyperventilation. However, it was observed that a little more feed settled in the quiescent zones of tanks stocked at 200% and 300% PCA than before the increase in water temperature.

Table 5.4 lists growth performance of the fish reared at the three stocking densities. Fish initially stocked within the treatments were chosen to provide a uniform TSA. There were no significant differences ($p > 0.05$) between treatment means for W, TL, TSA, BSA, and CV at the start of the experiment. Mean W were significantly different for the three treatments for Weeks 8 ($p < 0.05$) and 10 ($p < 0.05$), with the mean W for the 100% PCA treatment being greater than that for 200% PCA treatment, which was in turn greater than that at 300% PCA (Table 5.4). Significant differences ($p < 0.05$) between treatments were seen at Week 8 for TL, TSA, BSA, and CV (Table 5.4). Coefficient of variation (CV) increased significantly ($p<0.05$) from the start of the experiment to Week 10 among the three stocking densities. However, there was not clear trend in terms of the evolution over time of CV differences between the three stocking densities. In fact, although CVs were significantly different ($p < 0.05$) among stocking densities at Week 8, no differences were found at Week 10 (Table 5.4)

Table 5.4.- Summary of data from California halibut reared at three stocking densities for Week 0 (Initial), Week 8 (Final), and for selected parameters on Week 10 (Final at Week 10). A different letter in a row indicates significant differences ($p < 0.05$).

	Stocking density as PCA (%)					
	100		200		300	
	Average	SD	Average	SD	Average	SD
W (g)						
Initial	11.8	0.4	11.1	0.2	11.2	0.4
Final	23.9a	1.0	20.9b	0.4	19.0c	0.7
Final at Week 10	28.5a	1.6	24.4b	0.6	21.2c	0.6
TL (cm)						
Initial	10.4	0.03	10.3	0.1	10.3	0.1
Final	12.9a	0.1	12.5b	0.1	12.1c	0.2
TSA (cm^2/fish)						
Initial	33.3	0.3	33.0	0.6	32.9	0.4
Final	51.2a	1.2	48.1b	0.6	44.6c	1.2
BSA (cm^2/fish)						
Initial	21.7	0.5	21.5	0.4	21.4	0.4
Final	33.3a	0.2	31.9b	0.4	29.5c	0.8
CV (%)						
Initial	12.7a	2.3	12.9a	3.3	11.9a	1.8
Final	26.4a	1.8	23.0b	1.1	26.7a	1.1
Final at Week 10	29.4a	2.6	25.8a	3.6	30.1a	2.3
PCA (%)						
Initial	106	1	209	3	311	4
Final	163	4	302	7	421	12
Final at Week 10	165	6	311	5	425	8
Increment (%)	56		49		37	
BFA (kg/m^2)						
Initial	3.8	0.1	7.1	0.1	10.6	0.4
Final	7.6	0.3	13.3	0.2	17.9	0.6
Final at Week 10	9.1	0.5	15.5	0.4	20.0	0.6
Increment (%)	140		118		89	

SD = Standard deviation; Increment = [((final at week 10)-(initial))/(initial)] * 100

At the end of the experiment the largest fish were those stocked at 100% PCA, and the smallest those stocked at 300% PCA. In fact, fish stocked at 100% PCA by Week 10 increased up to 56% in PCA, against 49% and 37% for stocking densities at 200% PCA and 300% PCA, respectively (Table 5.4). In addition the increase in BFA at Week 10 was 140%, 118%, and 89% for the 100% PCA, 200% PCA, and 300% PCA, respectively (Table 5.4). On the other hand, growth in W over time was represented well by exponential curves, accounting for more than 99% of the variation in mean W among weeks (Fig 5.7).

Averages PWG, SGR (%/day), TSAG (%), and BSAG (%) for California halibut juvenile were affected significantly by stocking density (Table 5.5). A significant decrease ($p < 0.05$) in PWG, SGR (%/day), TSAG (%), and BSAG (%) were observed as density was increased. PWG decreased from a maximum of 103.5% in fish initially stocked at 100% PCA to 69.5% at a density of 300% PCA. SGR (%/day) decreased from a maximum of 1.18% / day in fish initially stocked at 100% PCA to 0.88% / day at a density of 300% PCA. TSAG (%) decreased from a maximum of 53.9% in fish initially stocked at 100% PCA to 35.5% at 300% PCA. BSAG (%) decreased from a maximum of 53.4% in fish initially stocked at 100% PCA to 37.7% at 300% PCA. The increase in TSAG and BSAG for fish stocked in the 100% PCA treatment was similar (~53%), but the ones stocked within the higher density treatments (200% and 300% PCA) showed slightly more growth in BSAG than in TSAG (Table 5.5).

The BG (g) and FE (g biomass / g feed) of California halibut juveniles were

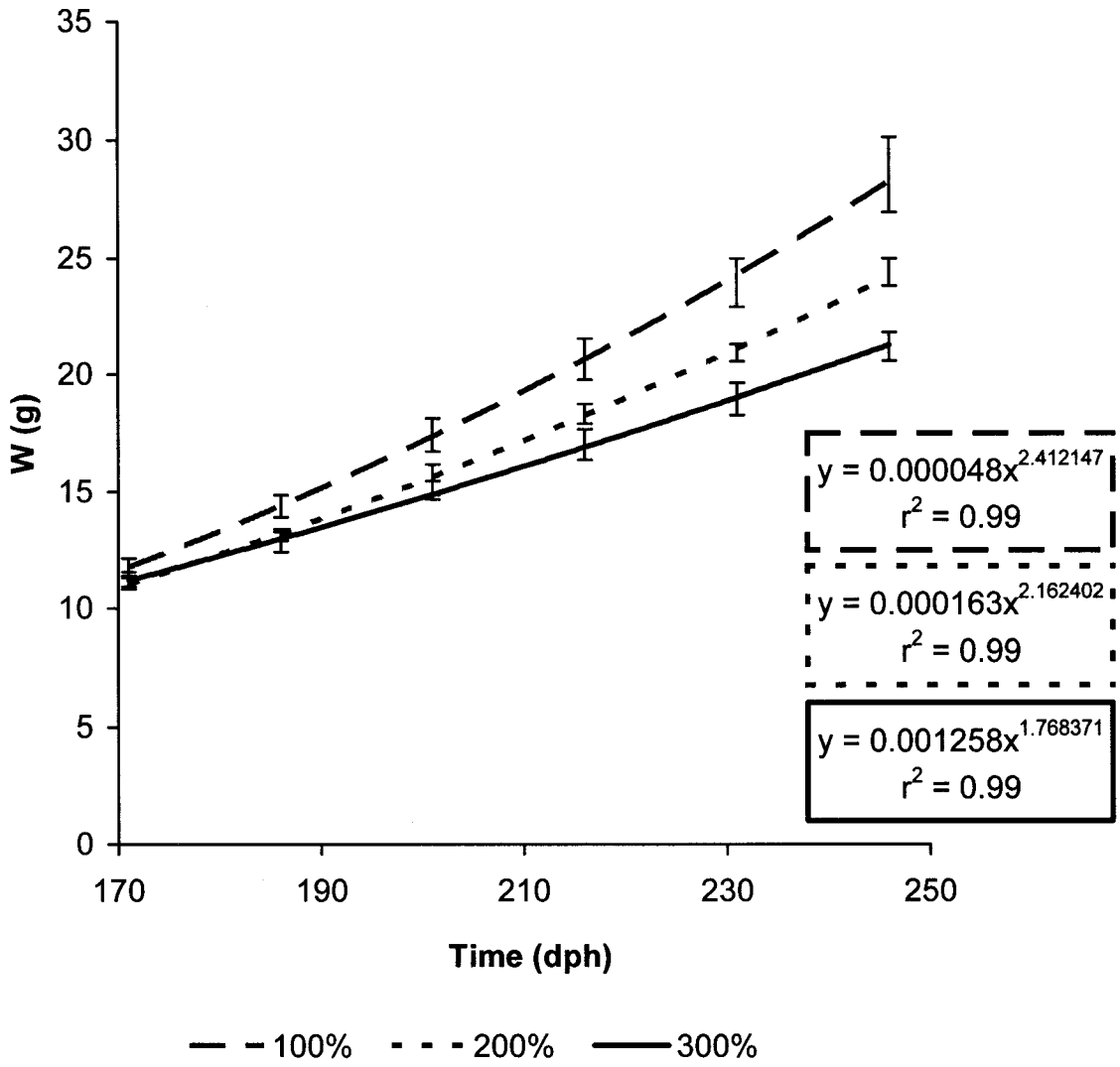

Figure 5.7.- Mean mass (W) of California halibut juveniles reared at three densities during ten weeks. Vertical lines represent standard deviations. In the equations above the term y is for W (g) and x is for time (dph).

Table 5.5.- Fish growth and feed efficiency data for California halibut reared at three stocking densities for eight weeks (mean value ± standard deviation). Values followed by a different letter on the same row are significantly different ($p < 0.05$).

	Stocking density (%)					
	100		200		300	
	Average	SD	Average	SD	Average	SD
PWG (%)	103.5a	3.8	88.4b	3.4	69.5c	6.4
SGR (%/d)	1.18a	0.03	1.06b	0.03	0.88c	0.06
TSAG (%)	53.9a	3.9	45.9b	1.4	35.5c	2.7
BSAG (%)	53.4a	2.3	48.2a	1.6	37.7b	2.8
BG (g)	243.6a	14.2	314.3b	10.7	279.6ab	22.4
FE (g biomass / g feed)	1.22a	0.03	1.10b	0.02	0.89c	0.06

SD = Standard deviation.

affected significantly by stocking density (Table 5.5). The largest BG was observed for the 200% PCA treatment, which was significantly different ($p < 0.05$; Table 5.5) from that of fish stocked at 100% PCA, but not from that of the ones stocked at 300% PCA. A significant decrease ($p < 0.05$) in FE occurred as stocking density was increased, from a maximum of 1.22 g/g in fish initially stocked at 100% PCA to 0.89 g/g at 300% PCA.

5.3.3.- Experiment 2. Fish growth in raceways and tanks

This experiment was terminated at Week 6 (March 1, 2003) due to oxygen problems within raceways stocked at 200 and 300% PCA. Growth data for California halibut juveniles reared at three stocking densities in different types of culture vessel are shown in Table 5.6. Fish were stocked at the same BFA in both vessels types, but water depths were different, resulting in different volumetric densities.

The W at Week 6 was not significantly different for the two culture vessel types ($p > 0.01$; Table 5.6). Therefore the null hypothesis cannot be rejected at individual significance level 0.01, and it is not possible to conclude that there is a difference in W between tanks and raceways. However, all W at Week 6 were significantly different for the different stocking densities ($p < 0.01$; Table 5.6). Consequently, the null hypothesis can be rejected and it can be concluded that there is a difference in W at Week 6 between stocking densities. A significant decrease ($p < 0.05$) in W was observed as stocking density was increased for both types of vessel (Table 5.6).

Table 5.6.- Growth summary and observed volumetric density for California halibut juveniles reared during six weeks at three stocking densities within two types of culture vessels. Tank depth was 6 cm and raceway depth was 3.6 - 4.5 cm.

	Stocking density (%)					
	100		200		300	
	Average	SD	Average	SD	Average	SD
Raceways						
W (g)						
Initial	11.2	*	11.7	*	11.0	*
Final	20.6	*	20.5	*	18.2	*
BFA (kg/m^2)						
Initial	3.9	*	7.5	*	11.1	*
Final	7.1	*	13.1	*	17.5	*
Volumetric density (kg/m^3)						
Initial	101	*	165	*	228	*
Final	181	*	286	*	359	*
Tanks						
W (g)						
Initial	11.8	0.4	11.1	0.2	11.2	0.4
Final	20.7	0.9	18.3	0.4	17.0	0.7
BFA (kg/m^2)						
Initial	3.7	0.1	7.1	0.1	10.6	0.4
Final	5.6	0.2	9.8	0.4	14.2	0.4
Volumetric density (kg/m^3)						
Initial	62.5	1.9	117.5	1.9	176.0	5.9
Final	109.8	4.7	193.9	4.5	267.6	10.3

* There was no SD for raceways since they were not replicated.

5.4.- DISCUSSION

Water velocities within the tanks and raceways induced swimming velocities which were less than 1.0 bl/s. In Chapter IV it was concluded that swimming velocities within the range used in these experiments does not affect the growth performance of California halibut juveniles.

5.4.1.- Water quality.

Salinity varied between 24.5 and 32.0 g/L and temperature between 19.4 and 25.7 °C. There is a single published work looking at effects of salinity and temperature on growth of California halibut. According to Madon (2002) growth rates for small California halibut (11.8 to 17.2 cm TL) was not different for salinities of 17-34 g/L at temperature of 20-28 °C. Therefore, ranges of salinity and temperature (Fig. 5.5) experienced by the fish (average sizes between 10 and 13 cm TL; Table 5.4) in the present work were within the range determined by Madon (2002) and therefore as were not expected to have a negative effect on growth performance.

The TAN was below 1 mg/L most of the time, but had some peaks that reached 5.5 mg/L on one occasion. At the time of the peak TAN concentration, salinity was 30 g/L, temperature 25.3 °C, and pH 7.22, resulting in an unionized ammonia N of 0.029 mg/L. There are a few reports on the effect of TAN on flatfish performance. It was found for turbot (13 to 104 g fish) that levels up to 13

mg TAN/L (34.5 g/L salinity; 17 °C; pH 7.9; 6 mg O_2/L; 0.568 mg/L unionized ammonia N) can be safe for its culture (Person-Le Ruyet at al., 1997). Alderson (1979) studying turbot (2.3 g fish) and Dover sole (0.6 to 6 g fish) found that concentrations up to 7 mg TAN /L (34 g/L salinity; 16 °C; pH 6.9 to 7.9; 0.034 to 0.330 mg/L unionized ammonia N) did not affect growth. Chang and Yo (1988) and Yang et al. (1992) reported that no adverse effect was observed in the growth of hirame at TAN concentrations between 0.07 and 0.56 mg/L in a closed circulation system. In general, TAN values observed in this research were lower than those reported in the literature, which were considered to be not growth limiting for other flatfish.

Nitrite concentrations were usually less than 0.6 mg NO_2-N/L but increased to 0.9 mg NO_2-N/L at the end of the study. Reports on the effect of NO_2-N on flatfish include one for hirame, where it was found that concentrations between 0.006 and 0.33 mg/L have no adverse effect on fish growth (Chang and Yo,1988). The literature on nitrite toxicity on marine fish is very scarce and focuses mainly on salmonid species (Handy and Poxton, 1993). Furthermore, different parameters like exogenous chloride and oxygen concentrations, species, growth phase, pH, and exposure time affect the tolerance of an organism to nitrite (Lewis and Morris, 1986; Bartlett and Neumann, 1998). However, several authors have recommended a safe level of 0.5 mg NO_2-N/L for mariculture practices (Blancheton, 2000; Blanchard et al., 2003). In conclusion, the nitrite values

observed in this research were sometimes over the recommended value, but there is not enough evidence to indicate that fish physiology was affected.

Alkalinity values were 2.4±0.3 meq/L and its daily variation was related with nitrification activity (Loyless and Malone, 1999; Timmons et al., 2001). Daily additions of sodium bicarbonate helped to keep the alkalinity between 1.5 and 2.8 meq/L (Hirayama, 1970; Siddall, 1974; Hirayama et al., 1988; Kaiser and Schmitz, 1988; Timmons et al., 2001). The daily addition of sodium bicarbonate also helped to maintain a stable pH level between 6.99 and 7.70. The alkalinity and pH values recorded during the ten week experiment were within safe recommended levels.

There is an extended literature about the effect of dissolved oxygen on flatfish physiology. For juvenile flounder, *Platichthys flesus*, the critical oxygen saturation causing a decrease in feeding efficiency was 30% (Tallqvist et al., 1999). For sole a decrease in activity was recorded at an oxygen saturation of 40% (Van der Thillart et al., 1994). A decrease in growth for plaice and dab is reported at 50% and 30% oxygen saturation, respectively, and a reduced frequency of feeding for plaice occurred at 30% oxygen saturation (Petersen and Pihl, 1995). Oxygen consumption for turbot was linear over the range of 60-100% of saturation for temperatures within the 7-16 ^{o}C range (Brown et al, 1984). The stocking density trial carried out in the raceways was terminated at the end of Week 6 (March 1, 2003) due to a drop of dissolved oxygen in the raceway effluent to less than 3

mg/L or 43% of saturation. This oxygen drop resulted from a combined effect of lower dissolved oxygen content in the influent culture water due to the temperature rise, a high stocking density in the raceways (200% and 300% PCA), the amount of feed offered, and California halibut physiology.

Fish stocked in the tanks at 200% and 300% PCA did not eat all the feed offered between Weeks 8 and 10. The increase in pellets left in the quiescent zones of the tanks loaded at 200% and 300% PCA started when the stocking density reached 283% PCA and 397% PCA, respectively. California halibut reared in the tanks had a drop in SGR during the last two weeks of experimentation, regardless of the tank stocking density. A reduction of feeding activity and a drop in SGR can indicate a possible stressful condition.

5.4.2.- Experiment 1. Morphometrics and growth in tanks

Morphometric analysis was planned originally for a period of ten weeks, but a mechanical problem with the chiller resulted in an increase of the water temperature from 22.1±0.6 °C to 23.3±0.6 °C at the end of Week 6. Therefore, it was decided to do a morphometric analysis at the next scheduled sampling (beginning of Week 8). Madon (2002) who experimented with California halibut juveniles of similar sizes to the ones used in this research did not detect differences in growth for temperatures ranging from 20 to 28 °C.

Results from Experiment 1, which began with California halibut juveniles with an initial biomass of ~11 g and a size of ~10 cm TL, indicated that stocking density affects their growth and morphometrics during a period of eight weeks. In this instance, fish stocked at 100% PCA (final 163% PCA) grew larger than those stocked at 200% PCA (final 301% PCA) and 300% PCA (final 420% PCA) treatments (Table 5.4). Fish held at the lowest densities had the largest SGR (Table 5.5). Most studies on flatfish stocking densities have found that higher densities result in lower growth. Turbot, with initial biomass of 8.62 g/fish, were stocked at four different densities (10%, 14%, 17%, and 21% PCA), and after 45 days fish reared at the higher stocking densities (final density 149.3% PCA) showed significantly slower growth rates than those held at lower densities (final density 67.4% PCA) (Irwin et al, 1999). Hirame, with initial biomass of 42 g/fish, were stocked at five different densities (33%, 50%, 100%, 200%, and 300 % PCA), and after 35 days of experimentation it was found that fish at the highest stocking density (final density 460% PCA) showed a significantly slower growth rate than those held at lowest densities (final density 45% PCA) (Jeon et al., 1993). Summer flounder with initial biomass of 7.8 g/fish, were stocked at three different densities (100%, 150%, and 200 % PCA), and after 58 days fish reared at the highest stocking density (final density 493% PCA) showed significantly slower growth rates than those held at the lowest density (final density 355% PCA) (King et al., 1998).

Feed efficiency was affected when California halibut were stocked at higher densities as has been described for hirame (Jeon et al., 1993). In this study, a considerable amount of feed was found in the quiescent zone of tanks stocked at 200% and 300% PCA starting on Week 8. There are reports that with increasing stocking density, an increase in aggressive behavior, growth, and feeding efficiency was observed for the dominant fish while stress, fin damage, and metabolic rates increased in subordinate fish (Refstie and Kittelsen, 1976; Purser and Hart, 1991; Canario et al., 1998). A study done with turbot did not find differences on growth nor aggressive behavior between equally sized or mixed animals (Sunde et al., 1998). Fish stocked in the present study were pre-selected by size and only one case of damaged tail was observed in each of the 300% PCA replicates. Bjornsson (1994) found that for Atlantic halibut more of the feed consumed was used for metabolism than for growth with increasing stocking densities. So it is likely that an increase in metabolic rate, which was not measured, at higher stocking densities might have been the cause for the feed efficiency and growth reduction found here with California halibut.

A direct effect of stocking density on growth variability has been reported by a number of authors (Andrews et al., 1971; Irwin et al, 1999; Lambert and Dutil, 2001). The relative size difference between members of a population usually increases with increasing stocking density due to social interactions, development of hierarchies, and establishment of territorial borders (Lambert and Dutil, 2001). Furthermore, an increase in the CV for fish biomass within a

population is considered to be indicative of the establishment of hierarchies (Jobling, 1994; Irwin et al, 1999; Lambert and Dutil, 2001). In this study, the CV increased significantly for all groups of California halibut during the 10 weeks of experimentation, indicating that behavioral interactions were affecting the growth rates at all stocking densities analyzed. However, there was not clear trend in terms of the evolution over time of CV differences among stocking densities. The CV for turbot was found to increase more rapidly in groups held at higher stocking densities (Irwin et al., 1999). It was also found that CV for turbot increased with increasing size of fish both for graded and ungraded groups (Sunde et al., 1998).

Average W and SGR were greater during the whole experimental period for fish stocked at 100% PCA (Table 5.4; Fig. 5.7; Table 5.5). According to Madon (2002), temperatures recorded in this experiment should not have affected the growth rate of California halibut. The TAN and nitrite levels were within the ranges described as safe for flatfish culture (Alderson, 1979; Person-Le Ruyet et al., 1987; Blancheton, 2000; Blanchard et al., 2003). No studies have been done to understand the relationship between oxygen saturation levels and growth performance for California halibut. Fluctuations in oxygen saturation below optimal levels may lead to appetite reduction and growth depression, and in the case of flatfish it seems to be a factor that is species-specific with reported minimum values between 30 and 60% (Brown et al., 1984; Van der Thillart et al., 1994; Petersen and Pihl, 1995; Tallqvist et al., 1999).

5.4.3.- Experiment 2. Fish growth in raceways and tanks

Small rectangular tanks were used in triplicate to set up a density experiment. The tanks had different widths depending on the stocking density treatment. The volume of the rearing unit has been reported as affecting fish growth (Ewing et al., 1998; Wexler et al, 2003). The surface area of the rearing unit has also been reported as affecting crustacean growth (Aiken and Waddy, 1978; D'Abramo et al., 2000) and non-flatfish bottom dwelling fish (Kaiser et al., 1995). Hence three raceways were stocked at the same densities (100%, 200%, and 300% PCA) and used as controls against the experimental tanks.

Results observed here for California halibut juveniles reared at three densities in small tanks agree well with results from larger vessels (Table 5.6). Raceways, which were 5.6 times larger, 2-3 times wider, and stocked with 9 to 16 times more biomass than tanks had similar growth results. The volumetric densities were higher for raceways than for tanks at the same stocking density (PCA). From the results, it appears that surface area available is more important than volume for the intensive culture of California halibut. Similarly, turbot and Atlantic halibut juveniles cultured in shallow raceways have growth rates equal to or better than those reared in deep tanks, for the same fish size and temperature regime (Oiestad, 1999). In this project California halibut juveniles were reared in the raceways up to 360 kg/m^3 (fish stocked at 300% PCA), but better growth rates were obtained for fish stocked at 100% PCA, which reached 181 kg/m^3 in six weeks. Volumetric densities in this research (Table 5.6) are larger than those

reported for turbot with a maximum of 75 kg/m^3 (Irwin et al., 1999; Martinez-Tapia and Fernandez-Pato,1991), Arctic charr with 50 kg/m^3 (Baker and Ayles, 1990), and commercial salmon farms with 30 kg/m^3 (Pennel and McLean, 1996).

5.4.4.- Stocking density projections

The practical implications of the present results are that California halibut can be grown in shallow raceways at a relatively high stocking density without significantly compromising growth rate and survivability. In the present research California halibut reared at a high density in a recirculating system at ~22 °C and fed with salmon pellets were heavier than those reported by Innis (1980).

The results for juvenile California halibut suggest that the stocking density effect on growth becomes evident after certain threshold level. Initial stocking densities of 100% PCA will produce a better individual fish growth over time than the other stocking densities tested. However, stocking fish at 200% PCA will yield a higher total biomass increase (Table 5.6), hence improving the fish production (kg/m^2 per year) of tank rearing in land-based farms. Fish should be split when stocking density reaches a maximum targeted PCA to reduce the biomass per area and prevent a significant reduction in SGR.

The biomass of fish per area (BFA) can be estimated by knowing W and the corresponding TSA, which ultimately will be compared against tank bottom available for culture. The TSA can be measured for flatfish by using image

analysis (Appendix A.2). For California halibut, TSA can be estimated using Eq 5.1. Once having W (g) and TSA (cm^2), an equation can be written that relates fish biomass (W) with stocking density:

$$BFA = \left(\frac{W}{TSA}\right) * \kappa \qquad \text{(Eq. 5.3)}$$

where, k = conversion factor (10) to express g/cm^2 as kg/m^2:

The ratio of W to TSA (Eq. 5.3) is equal to the BFA for a 100% PCA. To express a larger PCA another factor has to be incorporated. The stocking factor (β) will be 1 for 100% PCA, 2 for 200% PCA, and so on. The final expression will be:

$$BFA = \beta * \left(\frac{W}{TSA}\right) * \kappa \qquad \text{(Eq. 5.4)}$$

Increases in PCA will result in the corresponding rises in BFA as shown in Fig. 5.8. The graph does not represent growth, merely the biomass of fish stocked per surface area for a given fish size (W) and desired PCA. Ultimately, the optimal stocking density from the point of view of a fish farmer will depend on capital cost per unit of rearing area, availability and cost of juveniles, market price and size of fish.

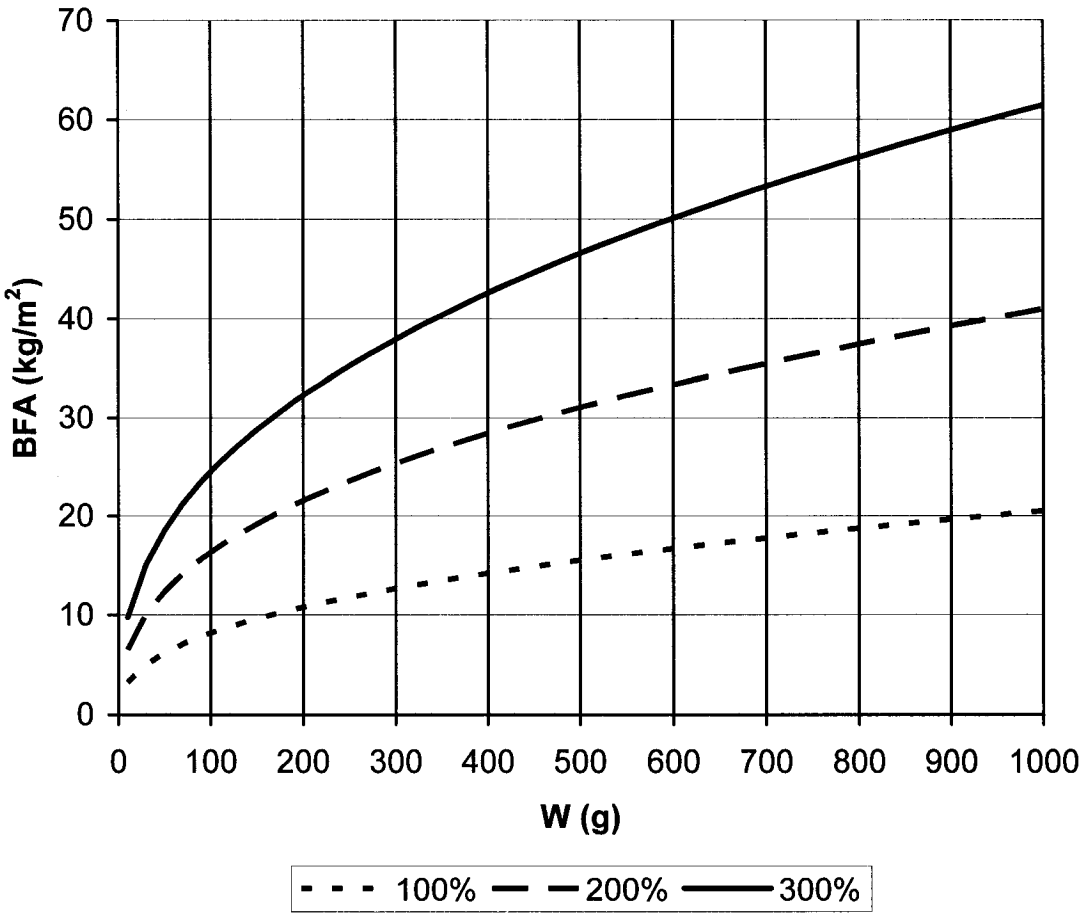

Figure 5.8.- Projected stocking density of California halibut corresponding to 100%, 200%, and 300% PCA related to biomass of fish.

5.5.- CONCLUSIONS

The most relevant conclusions from the present chapter are:

a) Growth of California halibut decreased as stocking density was increased from 100% to 300% PCA. Nevertheless, a larger biomass gain and little mortality indicate that California halibut can be reared at relatively high initial stocking densities of up to 200% PCA.

b) Maximum SGR's were obtained when California halibut juveniles were initially stocked at 100% PCA both for tanks and raceways.

c) Maximum BGs were obtained when California halibut juveniles were initially stocked at 200% PCA.

d) The results of this study could be used advantageously to plan yield-maximizing farming strategies for on-growing juvenile California halibut.

5.6.- FURTHER RESEARCH

Further studies are recommended in the following areas:

- Determination of the effects of TAN and nitrite on the growth and survival of California halibut under culture conditions.

- Determination of the effects of oxygen saturation on growth and survival of California halibut under culture conditions.

- Studies on how water quality varies within a culture tank for different fish stocking densities and for a variety of tank configurations.

- Studies on how growth is affected if tanks are stocked with graded and ungraded groups.

CHAPTER VI

OXYGEN CONSUMPTION RATES OF CALIFORNIA HALIBUT JUVENILES UNDER CULTURE

6.1.- INTRODUCTION

Successful operation of fish culture systems requires regulation of dissolved gas levels within acceptable limits. Fish oxygen requirement is a fundamental design variable of aquaculture system design and management, as it is the basis for determining water flow rates for sustaining stock (Timmons et al., 2001). High fish stocking densities are often limited by the rate of water flow through the rearing unit when the latter is used as the primary source of oxygen (Westers and Pratt, 1977; Fivelstad, 1988; Lawson, 1995). Oxygen supplementation may be used to increase the allowable fish loading or rearing densities for a given water flow rate (Colt et al., 1991). Oxygen uptake and concentration tolerance must be determined to be able to calculate flow rates using mass balances (Liao, 1971). Oxygen uptake by fish has been measured through metabolism studies under laboratory and farm conditions (Klein-MacPhee, 1979; Wood et al., 1979; Brown et al., 1984; Mallekh et al., 1998).

Metabolism studies on flatfish have been done in laboratory conditions with the use of respirometry chambers (Klein-MacPhee, 1979; Wood et al., 1979; Priede

and Holyday, 1980; Honda, 1988; Winger et al., 1999). However it has been demonstrated (Muller-Feuga et al., 1978; Bergheim et al., 1993; Fivelstad et al., 1999) that there are large differences between the oxygen consumption determined in the laboratory and field data obtained at a fish farm (Brown, 1980). The influence of variables such as biofouling growing on tank walls (Tudor, 1999), tank shape and stocking density (Bergheim et al., 1993), photoperiod length (Waller, 1992; Imsland et al,. 1995), fish activity and feeding level (Fivelstad et al., 1999), water flow rates (Brett, 1964; Smith et al., 1971; Christiansen et al., 1991), as well as stress associated with daily husbandry may be important factors contributing to the oxygen requirements in a fish farm, which are missed in laboratory studies (Brown et al., 1984; Mallekh et al., 1998). Hence studies of oxygen consumption in traditional respirometers under laboratory conditions may misrepresent the real oxygen needs under culture conditions (Brown et al., 1984; Forsberg, 1994; Thomas and Piedrahita, 1997).

The California halibut is a commercially important fish in the Pacific coastal waters of California (USA) and Baja California (Mexico). There is a lack of knowledge regarding the oxygen consumption rates of California halibut juveniles under intensive farming conditions. Therefore, the present study of the oxygen consumption of California halibut was conducted in open respirometers under farm-like conditions. This study was carried out to determine the oxygen consumption and the diel pattern of oxygen consumption of California halibut

juveniles between 3.2 and 165.6 g reared in a recirculating system under farm-like conditions.

6.1.1.- Literature review

Respirometry studies can be performed under farm-like conditions (Brown et al., 1984; Mallekh et al., 1998), where a fish tank is used as a closed or open respirometer (Brown, 1980). Under farm-like conditions, fish are exposed to light cycles and management routines that affect directly or indirectly fish growth, metabolic rates, and diel cycles (Brown et al., 1984; Haijin et al., 1997; Mallekh et al., 1998).

6.1.1.1.- Metabolic rates

There are three general metabolic states for which oxygen consumption has been defined: standard, routine, and active metabolism (Fry, 1957; MacIsaac et al., 1996). The standard rate describes when there is no spontaneous activity, the routine rate is when spontaneous activity occurs, and active rate is induced by forcing the fish to swim. In laboratory conditions usually standard or active metabolism states are studied (Mallekh and Lagardere, 2002). Under farm conditions fish typically present a routine metabolism state (Brown, 1980; Brown et al., 1984; Waller, 1992; Forsberg, 1994; MacIsaac et al., 1996; Thomas and Piedrahita, 1997).

6.1.1.2- Respirometry in open or flow-through systems

Oxygen consumption rates of fish species under farming conditions have been measured using culture tanks as open respirometers (Brown et al., 1984; Hallaraker et al., 1995; Imsland et al., 1995; Thomas and Piedrahita, 1997, 1998; Fivelstad et al., 1999). Steffensen (1989) stated that the effectiveness of open respirometers is limited by the ratio of the water flow (Q, L/min) to volume of water (V, L) in the respirometer, which is the dilution factor (D, 1/min):

$$D = Q / V \qquad \text{(Eq. 6.1)}$$

Spoor (1946) reported that the lag between a change in oxygen consumption and a change in fish activity depends on D (Steffensen, 1989). The lag time will depend on the extent of mixing, the volume, and the water flow in the respirometer. The lag time decreases with increasing dilution rates (Steffensen, 1989). In most open respirometry studies, D varies from 0.5/min to 0.05/min, with a corresponding 99% response time of 9.2 to 92 min, respectively (Steffensen, 1989).

A mass balance equation can be used to determine fish oxygen consumption rate by measuring changes in dissolved oxygen concentration from the influent to the effluent of a rearing unit. The rate of fish oxygen consumption (g O_2/kg fish per d or mg O_2/kg fish per h) is a function of feeding rate, feed quality, water quality, and fish metabolic demands (Jobling, 1994). Oxygen consumption rate is typically expressed relative to a unit of feed (g O_2/ g feed) (Timmons et al., 2001).

In aquaculture systems operated at oxygen concentrations different from saturation, surface oxygen diffusion may occur in tanks open to the atmosphere (Atkinson et al., 1995; Gelda et al., 1996; Thomas and Piedrahita, 1997; Fivelstad et al., 1999). This oxygen transfer has to be accounted for within the general mass balance equation.

6.1.1.3.- Diel fluctuations

Most respirometry studies in flatfish have measured oxygen consumption over periods of 12 h or less (Brown et al., 1984; Honda, 1988; Kikuchi et al., 1990). However, variations in the metabolic rate of fish may result in significant fluctuations in oxygen concentration over a 24 h cycle. Oxygen concentrations below critical levels may cause severe stress in fish, leading to appetite reduction and growth depression (Carlson et al., 1980; Mallekh and Lagardere, 2002). Above this critical level most fish compensate for the decrease in oxygen concentration by increasing their ventilation rate (Watters and Smith, 1973; Dejours et al., 1977). For turbot it was found that oxygen consumption rate varied linearly over the range of 60-100% of saturation for temperatures within 7-16 °C (Brown et al, 1984). For juvenile common flounder oxygen concentration below a critical 30% of saturation caused a decrease in predation efficiency (Tallqvist et al., 1999). For sole a decrease in activity was recorded at an oxygen saturation of 40% (Van der Thillart et al., 1994). Decreased growth for plaice and dab was recorded at 50% and 30% oxygen saturation respectively, with a reduced

frequency of feeding for plaice recorded at 30% oxygen saturation (Petersen and Pihl, 1995).

Diurnal variations in oxygen consumption in aquaculture systems have been related to feeding activity for sockeye salmon (Brett and Zala, 1975), Atlantic salmon (Bergheim et al., 1991), rainbow trout (Wagner et al., 1995), white sturgeon (Thomas and Piedrahita, 1997), and sea bass (Tudor, 1999). Diurnal dissolved oxygen variations can be significantly reduced by increasing the number of feedings per day and by lengthening the daily feeding period (Imsland et al., 1995). Peak oxygen consumption demands on fish culture systems may exceed daily mean demands by a factor of two or more (Klein-MacPhee, 1979; Colt et al., 1991; Imsland et al., 1995; Forsberg and Bergheim, 1996; Jarboe, 1996; Haijin, et al., 1997). Hence, both maximum and mean oxygen consumption rates are necessary for the correct design of an aquaculture operation.

6.1.1.4.- Culture conditions

Under farm conditions, flatfish are stocked at high densities and a group effect on fish physiology is to be expected. Honda (1988) reported that oxygen consumption in hirame held singly was 11 to 17% greater than that of fish held in groups. Parker (1973) attributed this phenomenon to an interaction of a calming effect and a possible hydrodynamic effect. A calming role has also been shown for other fishes (Kanda and Itazawa, 1981; Umezawa et al., 1983).

In high density culture, flow rates may be adjusted to provide adequate oxygen levels. However, these flow rates may result in higher than desired water velocities (Brett, 1964; Smith et al., 1971; Christiansen et al., 1991). It has been reported that oxygen consumption rates in common flounder, common dab, and lemon sole increased with exposure to increased water velocities (Duthie, 1982). For California halibut juveniles, it was found in Chapter IV that water velocities under 1.0 body length per second (bl/s) did not affect growth rates negatively.

6.1.1.5.- Reporting oxygen consumption analysis

Brown (1980) and Steffensen (1989) listed some considerations when reporting oxygen consumption obtained from open respirometry:

a) It must be stated whether the data are derived from an average 24 h value, or when the fish have been fed their daily ration.
b) The diet must be specified in terms of its protein content, as increasing protein content increases oxygen consumption.
c) The condition of temperature, salinity and photoperiod under which the experiments were conducted must be specified, with particular reference to water current.
d) The stocking density under which the fish are kept should be given, as the subsequent effect on water quality (particularly CO_2) may have an effect on metabolic rate.

e) The dilution factor (D) in the respirometer has to be reported. As D affects the time response at which a change in oxygen consumption can be detected. A higher D will shorten the time response.

6.2.- EXPERIMENTAL DESIGN

The procedures followed to determine oxygen consumption in California halibut stocks held under farm-like conditions are described in this section. Fish were fed daily during 12 h within the 16 h light period. Respirometries were performed during 24 h cycles. Oxygen consumption rates were determined by mass balance calculations. A diel pattern of oxygen consumption was determined from the data recorded during the 24 h respirometry studies.

6.2.1.- Fish stock

Two generation groups, 2001 and 2002, of juvenile California halibut spawned from captive broodstock were used in this experiment. Fish from the 2001 generation provided average individual biomass between 100 and 170 g, and the 2002 generation provided average biomass between 2.8 to 21 g. The juveniles were reared intensively at the California Halibut Recirculating Hatchery located at The University of California, Davis. The recirculating system operated at a constant temperature of 22 ± 1 ^{o}C and salinity of 30 g/L (Gadomski and Caddell, 1991; Madon, 2002). Light was provided by overhead fluorescent tubes on a 16

L: 8 D (L=light; D=dark) photoperiod (Boeuf and Le Bail, 1999; Klokseth and Oiestad, 1999). Lights were on from 7:00 to 23:00 h.

6.2.2.- Experimental tank

The recirculating system contained four raceways; three of which were used for the respirometry studies (Appendix A.1). The raceways were 27.5 cm wide, 241 cm long and had a water depth of about 5 cm. A quiescent zone in the effluent section was 19 cm long. The water level was controlled by an external standpipe. Cylindrical nets (5 mm aperture) were placed within the raceways at the influent and effluent piping section to avoid fish escapement and to provide a frame of support for a dissolved oxygen sensor (Fig. 5.2; Fig. 6.1). Influent water entered under the water surface at the head of the tank.

6.2.3.- Water quality measurements

Influent and effluent water were monitored for oxygen concentration *in situ* at 1 min intervals with a dissolved oxygen sensor (Sensorex™ model DO6000), and 10 min averages were saved. Temperature was monitored with a copper-constantan thermocouple in a stainless steel probe (Omega®) placed within the head tank. Data from oxygen sensors and thermocouples were recorded with a datalogger (CR-21X, Campbell Scientific, Inc.). Data recorded with the datalogger were uploaded to a PC computer using PC208 W 3.3 datalogger support communication software (Campbell Scientific Inc.). Data were uploaded every week, and stored in a workbook in Microsoft Excel 2002 SP-2.

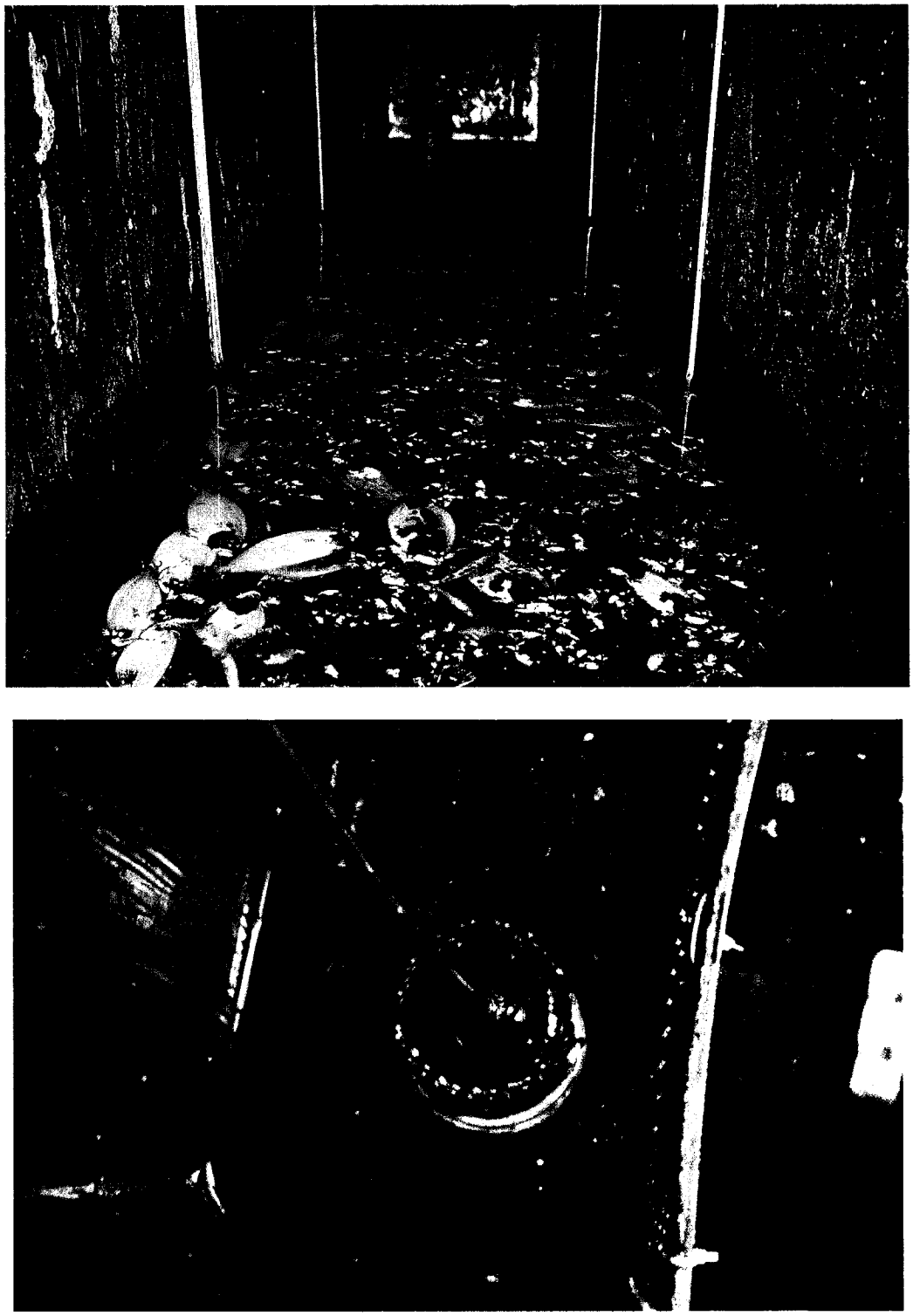

Figure 6.1.- DO probes in a raceway. Influent probe located at the head of the raceway (top picture). Effluent probe located in the effluent section of the raceway (bottom picture).

Dissolved oxygen (DO) probes (Sensorex™ model DO6000) were calibrated the day before starting an oxygen consumption test. For calibration all DO sensors were placed into the head tank (100 L), which was aerated with an airstone, for at least 3 hours.Saturation concentration oxygen for seawater, needed for probe calibration, was determined from values of atmospheric pressure, water temperature, and salinity (Colt, 1984). Local barometric pressures were obtained from the National Weather Service for the Sacramento International Airport, about 30 km from UC Davis (www.city.davis.ca.us/topic/weather.cfm). The DO saturation values calculated were used to adjust the probe readings in the calibration process. A minimum water velocity of ~5 cm/s (2 in/s) across the probe membrane is required for proper DO readings.

Total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) were measured with Hach™ reagents using a Hach™ Odissey spectrophotometer (Model DR/2500). The TAN was measured by the salycilate method (Hach™ method 8155) which is specific for seawater samples. Nitrite was measured by using the diazotization method (Hach™ method 8507) approved by USEPA for freshwater, seawater, and wastewater. The pH readings were done with a Fisher Scientific pH-meter Accumet (Model 50). Alkalinity was measured by titration (La Motte Chemical test kit, model WAT-DR). Salinity was measured with an YSI SCT meter (Model 33). TAN and nitrite were measured weekly during the morning before feeding, while pH, alkalinity, and salinity were taken daily at 9:00 AM. Daily additions of sodium

bicarbonate were needed to keep the alkalinity value above 2 meq/L (Timmons et al., 2001).

6.2.4.- Fish sampling

The fish in each raceway were weighed for total biomass, B, and counted every other week, and feeding ration was adjusted at this time. Oxygen consumption experiments were performed during three consecutive days just prior to a weighing. The absolute growth rate (AGR, g/d, Jobling, 1994) expression (Eq. 6.2) was used to estimate the fish biomass (B_{exp}, g) (Eq. 6.3) during each of the three oxygen consumption experiments:

$$AGR = (B_2 - B_1) / (t_2 - t_1) \qquad \text{(Eq. 6.2)}$$

where B = total fish biomass (g). Subscripts denote initial (1) and final (2) times

t = time (d). Subscripts are as for B

$$B_{exp} = B_1 + (AGR * (t_{exp} - t_1) \qquad \text{(Eq. 6.3)}$$

where t_{exp} = time period when the experiment was performed (d)

The B_{exp} and the mortality record were used to determine mean biomass (W, g) and culture density as a percentage of coverage area (PCA, %):

$$W = B_{exp} / N \quad \text{(Eq. 6.4)}$$

where N = number of fish within the raceway

$$PCA = 100 * (TSA * N) / A \quad \text{(Eq. 6.5)}$$

where TSA = average fish total surface area (cm^2)

A = tank surface area used for culture (cm^2).

PCA was estimated for the 2002 fish generation (<30 g) using a relationship between TSA and W (Appendix A.2; Fig. A2.4):

$$TSA_{2002} = 7.0801 * W^{0.6056} \quad \text{(Eq. 6.6)}$$

Similarly, a PCA for 2001 fish (>66 g) was also estimated using a relationship between TSA and W (Appendix A.2; Fig. A2.8):

$$TSA_{2001} = 11.224 * W^{0.5716} \quad \text{(Eq. 6.7)}$$

6.2.5.- Water flow rates

Water flow for each raceway was determined by measuring the time taken to fill a 15 L container. The procedure was repeated three times. Water flow measurements were made during the days chosen to perform the oxygen consumption experiment.

6.2.6.- Feeding and feeds

Feeding rates used in the present study were set according to the feeding protocol at the Hatchery and Nursery facilities (Table 6.1). Feed amount was set after each weighing, hence feeding ratio decreased slightly throughout the two weeks between weighings. Feed was distributed by automatic 12 h belt feeders (09:00 to 21:00) during the daylight hours (07:00 to 23:00) 7 d/wk.

Diets used were manufactured by Silver Cup® and EWOS® Canada Limited for salmonid species (Table 6.2). Silver Cup® fry pellets 1 and 2 mm were used to feed fish less than 7 g. The EWOS® pellets 2 and 3 mm were used to feed fish larger than 10 g.

6.2.7.- Model of dissolved oxygen in an open respirometer

Following a methodology described by several authors (Imsland et al., 1995; Thomas and Piedrahita, 1997; Fivelstad et al., 1999) oxygen consumption rates were determined from non-steady state mass balance calculations under farm-like conditions. The raceways were considered as flow through respirometers open to the atmosphere (Fig. 6.2).

Table 6.1.- Feed ration for California halibut as a percentage of fish biomass (%BW). Feed ration was pre-determined by the Recirculating California Halibut Hatchery Management Protocol.

W (g)	Feed ration %BW
3 – 6	3.0 – 2.5
6 – 25	2.0 – 1.0
25 - 75	1.5 – 1.0
75 – 200	1.2 - 0.5

Table 6.2.- Nutritional characteristics of feed used to grow California halibut, as supplied by the manufacturers.

Contents	SILVER CUP	EWOS
Crude protein	45 %	43 %
Crude fat	19 %	14 %
Crude fibre	3 %	2 %
Ash	12 %	9 %
Moisture	Less than 10 %	8 %
Vitamin A	10000 IU/kg	3000 IU/kg
Vitamin D3	500 IU/kg	3000 IU/kg
Vitamin E	250 IU/kg	150 IU/kg

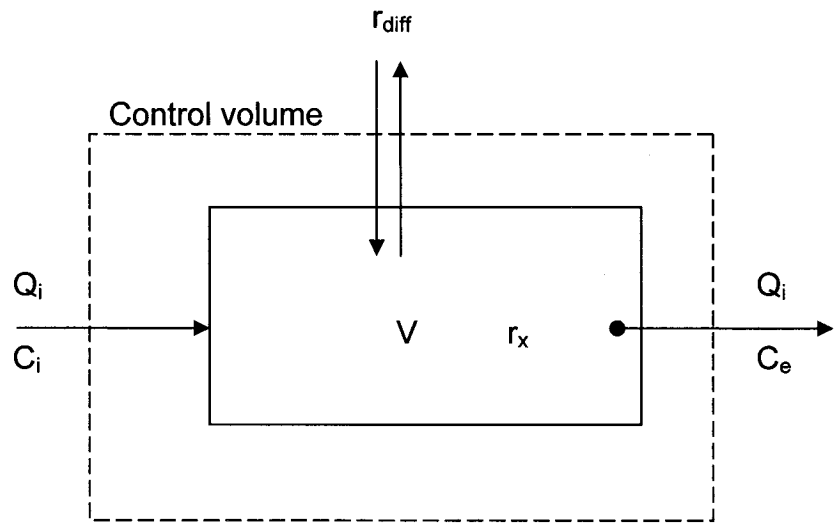

Figure 6.2.- Schematic diagram for mass balance analysis in a raceway. Terms are as defined in the text.

The dissolved oxygen mass balance applied to a raceway of constant volume (Fig. 6.2) can be described with (Thomas and Piedrahita, 1997):

$$\frac{dC}{dt}V = Q_i(C_i - C_e) + r_{diff}V + r_xV \qquad \text{(Eq. 6.8)}$$

where $\frac{dC}{dt}$ = change in concentration of dissolved oxygen in the raceway over a period of time (mg / L h)

V = raceway volume (L)

Q_i = flow rate of water entering and leaving the raceway (L/h)

C_i = dissolved oxygen concentration in the influent flow (mg / L)

C_e = dissolved oxygen concentration in the effluent flow (mg / L)

r_{diff} = rate of diffusion of oxygen between water and the atmosphere (mg / L h)

r_x = net rate of production of oxygen (mg / L h). A negative r_x indicates a consumption of oxygen

Brown et al. (1984) reported for turbot respirometry studies that the rate of decline in dissolved oxygen concentration was constant over the range 95-60% of oxygen saturation. Hence the inlet water flow rate, for each raceway, was adjusted the day before the beginning of a respirometry experiment to keep a minimum level of 5 mg/L of dissolved oxygen at the raceway outlet. Consequently, the flow was regulated to result in a ~28% reduction of the oxygen

content in the water running through a raceway. This operational condition assumed therefore that r_x was independent of dissolved oxygen concentration within the range of dissolved oxygen in the raceways.

Relative swimming velocities during the respirometry experiments were between 0.05 and 0.2 bl/s. It was found in Chapter IV that swimming velocities under 1.0 bl/s did not affect growth rates negatively.

Diffusion rates of oxygen across the water surface are a function of the difference between the saturation gas concentration and the concentration in the water. The r_{diff} was defined as negative if oxygen diffused from the water to the atmosphere. The rate of diffusion of oxygen was estimated by the Lewis-Whitman gas transfer model (Lawson, 1995):

$$r_{diff} = \frac{D}{\Delta}\frac{A}{V}(C_s - C_e) \qquad \text{(Eq. 6.9)}$$

where r_{diff} = oxygen transfer rate (mg/ L h)

D = oxygen diffusion coefficient (m^2 / h)

Δ = liquid film thickness (m)

A = area of gas-liquid interface (m^2)

V = volume of water in the raceway (m^3)

C_s = saturation concentration of oxygen (mg / L)

C_e = dissolved oxygen concentration in the effluent (mg / L)

The oxygen transfer coefficient ($K_L = D/\Delta$) was calculated from a characteristic thickness of the water-air interface layer of 100 μm (Tudor, 1999), and from an equation given by Richard (2003) for temperatures between 20 and 60 °C:

$$D = 4.601 * 10^{-6} * e^{T*2.252*10^{-2}} \qquad \text{(Eq. 6.10)}$$

where D = diffusion coefficient (m^2 / h)

T = temperature (°C)

6.2.8.- Calculation of oxygen consumption rates (r_x)

Oxygen consumption rates (r_x) were calculated from Eq. 6.8 using dissolved oxygen measurements made every minute and averaged and recorded every 10 min. The change in concentration over time (dC/dt) was calculated from the measured C_e values. The diffusion rate (r_{diff}) was estimated from Eq. 6.9. The consumption term was then calculated for the fraction of the hour using Eq. 6.8.

The rate of oxygen consumption measured in an open respirometer may include microbiological oxidation or biochemical oxygen demand besides fish respiration. The oxygen drop in an unstocked raceway was determined and it was described in section 6.2.10.1. This measurement constituted an estimation of r_x due to organism other than the fish. If the oxygen drop in an unstocked raceway is too small then it can be neglected from Eq 6.8 and it can be assumed that all r_x is due to the fish.

The rate of oxygen consumption may be expressed per unit of fish biomass per unit of time by dividing the consumption term by the fish biomass stocked in a given raceway (M, g O_2 / kg fish per h):

$$M = r_x * V / B_{exp} \qquad \text{(Eq. 6.11)}$$

Mean daily values of oxygen consumption (M_{day}, g O_2 / kg fish per d) were obtained by averaging the r_x values calculated from Eq. 6.8 for each 10 min interval:

$$M_{day} = \overline{M} * 24 \qquad \text{(Eq. 6.12)}$$

Oxygen consumption relative to the amount of feed given was estimated by assuming that the feed was distributed evenly during 24 h. The rate of oxygen consumption may be expressed per unit of feed by dividing the consumption term by the daily feed offered (M_F, g O_2 per h/ g feed per h):

$$M_F = r_x * V * c / (F/c') \qquad \text{(Eq. 6.13)}$$

where F = amount of feed offered per day (g/d).

c = constant to convert mg to g O_2 (1 g / 1000 mg)

c' = constant to convert h to d units (24 h/d)

Mean daily values of oxygen consumption ($M_{F\text{-}day}$, g O_2 / g feed) were obtained by averaging the M_F values:

$$M_{F-day} = \overline{M_F} \qquad \text{(Eq. 6.14)}$$

6.2.9.- Oxygen consumption rate and fish size relationship

The results were analyzed using the relationship between oxygen consumption rate and fish size proposed by Jobling (1994):

$$M_{day} = a * W^b \qquad \text{(Eq. 6.15)}$$

where M_{day} = daily average or daily maximum rate of oxygen consumption (g O_2 / kg fish d)

a and b are species specific constants

Microsoft ® Excel 2002 was used to find model parameters "a" and "b".

6.2.10.- Experimental protocol

All oxygen consumption experiments were carried out over 24 h and three times over three consecutive days. Hence oxygen consumption was tested in the same raceway with the same biomass during three consecutive days. Raceway surfaces were cleaned every morning between 8:00 h and 9:00 h. Leftover feed and feces, if any, were removed in the morning (9:00) and evening (17:00).

6.2.10.1.- Oxygen drop in an unstocked raceway

The model (Eq. 6.8) includes a term, r_x, to account for oxygen consumption which was assumed to be only due to fish metabolism. However, oxygen concentrations can vary markedly in aquaculture systems subjected to high biochemical oxygen demand (BOD) due to uneaten feed and decaying wastes which are only removed intermittently (Harris et al., 1999). In addition, microbial oxidation of organic matter or nitrification in the water or on tank walls may also consume oxygen (Tudor, 1999).

In an attempt at estimating the rate of oxygen consumption by processes other than fish respiration, r_x was estimated for a raceway without fish. The raceway, was not scrubbed as was the normal maintenance practice, and r_x measurements were made over several days.

6.2.10.2.- Oxygen consumption

Oxygen consumption experiments started on August 30, 2002 with Generation 2001 fish (110 to 166 g). The fish were fed commercial dry pelleted feeds (EWOS™ 3 mm) at a ratio of ~0.70 to 1.20 %BW. Experiments were performed over a three month period with fish stocked at densities between 94% and 287% PCA.

Oxygen consumption experiments continued on November 22, 2002, with Generation 2002 fish (2.8 to 12.5 g). The fish were fed commercial dry pelleted

feeds (Silver Cup™ 1 and EWOS™ Vita 2 mm) at a ratio of 3.00 to 1.20 %BW. Culture densities during this period were between 107% and 316% PCA.

6.2.11.- Experimental design and statistical analysis

Fish mass, stocking density and dissolved oxygen concentration were the main parameters recorded on the oxygen consumption experiments performed during three consecutive days. Water quality was similar for all tests.

6.2.11.1.- Oxygen consumption

In averaging data sets with more than one day of data, average set means have to be similar (Dean and Voss, 1999). The student's *t* test was used for statistical comparisons of replicate daily means (two tailed tests) of the oxygen consumption tests performed. No significant differences were found among any of the replicate means ($p > 0.05$), and the data for the different replicates were therefore averaged (Dean and Voss, 1999).

A one way analysis of variance was done (Dean and Voss, 1999), to study the fixed effect of fish mass on oxygen consumption rates of California halibut. The null hypothesis (H_o) is that there is no difference in daily mean and daily maximum oxygen consumption rates between fish of different mass. The corresponding alternative hypothesis (H_i) is that at least one level is different. Significances were analyzed using Tukey and Duncan's multiple range tests

(Dean and Voss, 1999). All statistical analysis was performed with SAS statistical software (release 8.02 Level 02M0)

6.2.11.2.- Diel pattern of oxygen consumption.

Diurnal fluctuations in oxygen demand for California halibut juveniles described above were examined. Significant differences were not found between any of the replicate means ($p > 0.05$), and the data set from three days were therefore averaged for each time of the day (Dean and Voss, 1999).

6.3.- RESULTS

The results section will present first the water quality in the recirculating system, followed by the oxygen consumption in unstocked and stocked raceways. Finally, hourly and daily oxygen consumption will be analyzed for all fish sizes tested.

6.3.1.- Water quality

Water quality parameters in the recirculating system were very stable during the experimental time. Mean salinity values were 30.6 ± 0.8 g/L and daily morning temperature were 21.56 ± 0.97 °C. The pH morning values were 7.69 ± 0.18. Alkalinity fluctuated between 1.6 and 3.6 meq/L with a mean of 2.7 ± 0.4 meq/L. The mean TAN was 0.06 ± 0.03 mg/L, with maximum of 0.12 mg/L and a minimum of 0.01 mg/L. The mean nitrite-N was 0.46 ± 0.22 mg/L, with a maximum of 0.82 mg/L and a minimum of 0.12 mg/L.

6.3.2.- Oxygen drop in an unstocked raceway

Influent and effluent dissolved oxygen concentration for the empty raceway was very similar during the ten days monitored (Fig. 6.3). Differences between influent and effluent dissolved oxygen were negligible when compared with the dissolved oxygen drop in a raceway stocked with fish (Fig 6.3).

The recirculating system was heavily loaded with fish until August 29. During the last three days most fish were removed from the system, leaving only 100 fish (total biomass of 11123 g) in the raceway being used as a control for the empty raceway. A substantial reduction in the fluctuations of the influent dissolved oxygen was achieved after removing the fish from the recirculating system, possibly due to a drop in BOD of the system water. Given these results the r_x from Eq. 6.8 is assumed to be due to fish metabolisms.

6.3.3.- Oxygen consumption by fish

Table 6.3 shows rearing conditions during the oxygen consumption measurements. Fish were held at densities between 94 and 389% PCA. Mean and maximum oxygen consumption by mass were significantly different among some of the groups of fish tested ($p < 0.05$). Overall, the tendency was a decrease in average oxygen consumption rate with an increase in fish biomass.

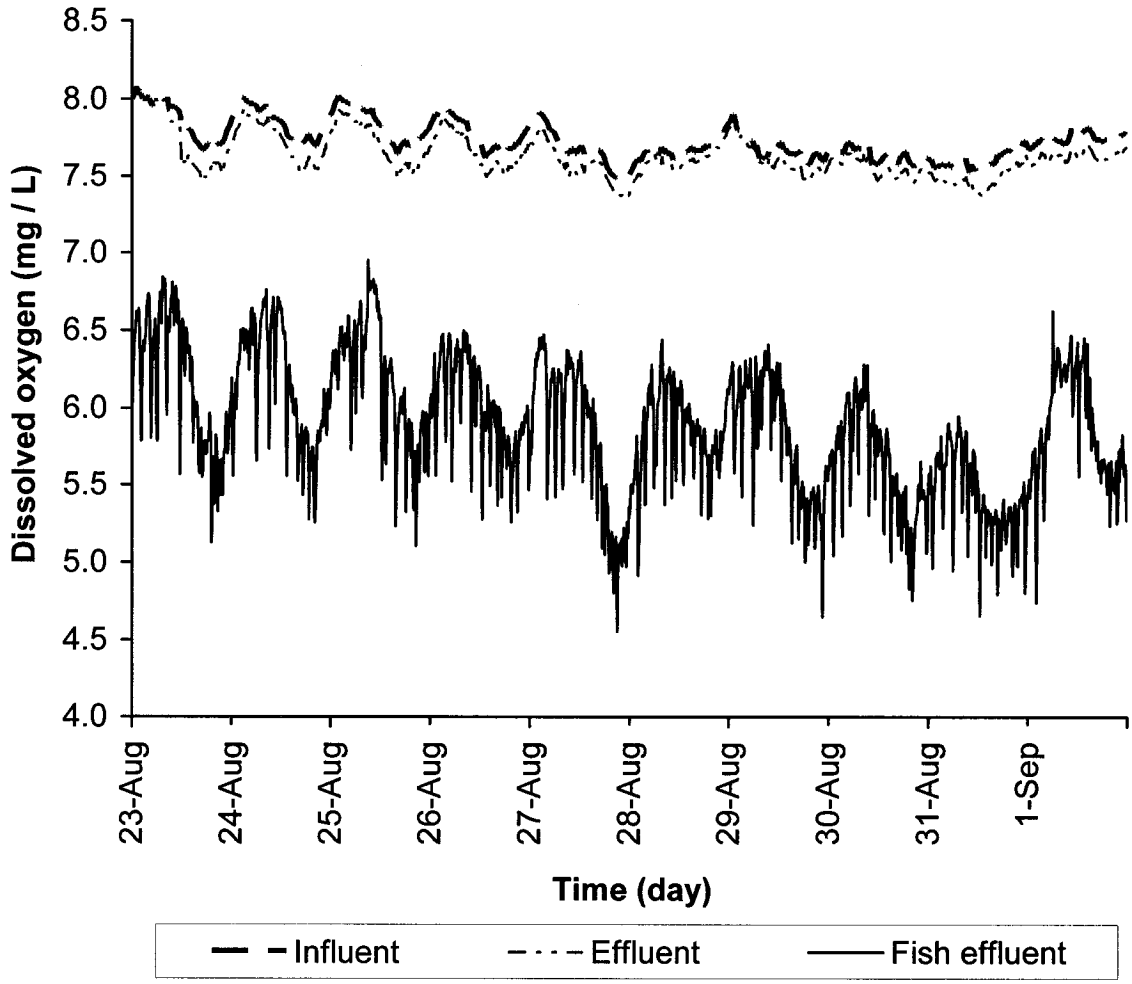

Figure 6.3.- Dissolved oxygen in an unstocked raceway. Both influent and effluent dissolved oxygen of an empty raceway are plotted along with the dissolved oxygen in the effluent from a raceway stocked with fish.

Table 6.3.- Oxygen consumption by California halibut juveniles. Data followed with similar letters within a column are not significantly different ($p > 0.05$). Data decrease in alphabetical order.

Feed	F	W	PCA	M_{day} (g O_2 / kg fish d)					M_F (g O_2 / g feed)				
Brand – size	%BW	g	%	Mean	SD	Max	SD	Max/Mean	Mean	SD	Max	SD	Max/Mean
Silver Cup – 1	3.0	3.2	209	11.4 b	0.3	13.8 b	0.8	1.21	0.43 k j h i	0.03	0.52 e f g	0.05	1.21
Silver Cup – 1	3.0	4.4	227	10.5 c	0.1	13.0 b	0.2	1.24	0.40 k j l i	0.01	0.50 f g	0.01	1.24
Silver Cup – 2	3.0	6.2	179	8.8 d	0.1	11.0 c	0.2	1.25	0.31 i	0.01	0.39 g	0.01	1.25
EWOS – 2	2.0	7.5	351	8.9 d	0.4	10.7 c d	0.5	1.20	0.49 g h i	0.02	0.59 e f g	0.02	1.20
EWOS – 2	2.0	9.2	389	7.7 e	0.6	9.4 e f	0.4	1.22	0.48 g j h i	0.04	0.59 e f g	0.03	1.22
EWOS – 2	1.2	11.8	316	7.7 e	0.3	9.7 d e	0.4	1.27	0.68 c	0.03	0.86 b c d	0.04	1.27
EWOS – 2	1.2	12.8	212	8.6 d	0.2	10.8 c d	0.5	1.25	0.79 b	0.02	0.99 b	0.04	1.25
EWOS – 2	1.2	12.5	107	12.4 a	0.2	16.1 a	1.5	1.30	1.08 a	0.01	1.40 a	0.11	1.30
EWOS – 2	2.0	14.2	243	7.4 e	0.6	9.4 e f	0.5	1.27	0.42 k j h i	0.03	0.53 e f g	0.03	1.27
EWOS – 3	0.7	110.5	270	4.1 g	0.2	6.3 l j	0.3	1.53	0.56 g f d e	0.04	0.86 b c d	0.03	1.53
EWOS – 3	0.7	113.3	275	4.5 g	0.7	6.9 h i	0.4	1.54	0.65 c d	0.10	1.00 b	0.06	1.54
EWOS – 3	0.7	113.8	189	3.9 g	0.6	5.5 j	0.4	1.41	0.57 g f d e	0.05	0.81 b c d	0.07	1.41
EWOS – 3	0.7	115.7	281	3.9 g	0.3	6.0 l j	0.2	1.54	0.57 g f d e	0.03	0.87 b c d	0.04	1.54
EWOS – 3	1.2	117.9	281	4.3 g	0.1	6.2 l j	0.3	1.44	0.38 k j l	0.01	0.54 e f g	0.03	1.44
EWOS – 3	1.2	120.1	195	6.0 f	0.9	7.7 g h	0.9	1.29	0.54 g f e	0.08	0.70 d e f	0.09	1.29
EWOS – 3	1.2	126.5	287	4.3 g	0.6	6.3 l j	1.1	1.46	0.37 k l	0.05	0.54 e f g	0.09	1.46
EWOS – 3	1.2	131.6	200	4.5 g	0.2	6.1 l j	0.4	1.37	0.38 k j l	0.02	0.52 e f g	0.04	1.37
EWOS – 3	0.7	132.4	94	4.2 g	0.8	5.6 l j	1.2	1.33	0.62 c f d e	0.12	0.82 b c d	0.19	1.33
EWOS – 3	0.7	133.0	296	4.1 g	0.2	5.79 l j	0.01	1.41	0.62 c d e	0.03	0.88 b c d	0.00	1.41
EWOS – 3	1.2	140.6	98	5.6 f	0.1	7.6 g h	0.3	1.37	0.49 g h i	0.01	0.68 d e f	0.03	1.37
EWOS – 3	0.7	141.6	208	4.6 g	0.9	6.0 l j	0.3	1.30	0.71 b c	0.15	0.93 b c	0.05	1.30
EWOS – 3	0.7	165.6	106	4.3 g	0.2	5.8 l j	0.6	1.40	0.69 c	0.03	0.92 b	0.09	1.40

In general, M_{day} was greater for fish with mass less than 4.4 g, and smaller for fish with mass larger than 110.5 g ($p<0.05$). An intermediate M_{day} was observed for fish between 6.2 to 14.2 g. Most M_{day} for fish over 110.5 g were not significantly different among themselves ($p < 0.05$), except for the 120.1 and 140.6 g fish, which had a higher oxygen consumption rate (Table 6.3). Maximum M_{day} values were between 20 to 30% greater than mean M_{day} values for fish under 14.2 g. Maximum M_{day} values were between 29 and 54% greater than mean M_{day} values for fish larger than 110.5 g.

6.3.4.- Oxygen consumption relative to the feed offered

Mean and maximum M_F were significantly different among the groups of fish tested ($p < 0.05$) (Table 6.3). Mean M_F values were between 0.31 and 1.08 g O_2/g feed, while maximum M_F ranged between 0.39 and 1.4 g O_2/g feed. In addition, M_F values increased with a decrease in the ration offered for fish with a similar average mass (11.8 to 14.2; 126.5 to 133.0; 140.6 and 141.6), independent of their stocking density (Table 6.3).

The highest mean M_F values for fish fed at 1.2%BW/day were for those weighing between 11.8 and 12.5 g. The smallest mean M_F values for fish fed at 1.2%BW/day were for those weighing between 117.9 and 140.6 g. The highest M_F values for fish fed at 0.7%BW/day were for those weighing between 141.6 and 165.6 g. The smallest M_F values for fish fed at 0.7%BW/day were for those weighing between 110.5 and 133 g.

For fish weighing 115.7 and 117.9 g the average M_{day} did not change with an increase of feeding ration from 0.7 to 1.2 %BW for fish stocked at 281 %PCA (Table 6.3). Maximum M_{day} did not vary either (Table 6.3). However, the corresponding mean and maximum M_F values were significantly different between the feeding ration offered ($p < 0.05$; Table 6.3), with the mean and maximum M_F values for fish fed at 0.7 %BW being larger than those for fish fed at 1.2 %BW. Calculated maximum M_F values were between 44 and 54% greater than average rates.

For fish weighing 132.4 (94 %PCA), 133.0 (296 %PCA), 126.5 (287 %PCA), and 131.6 g (200 %PCA) the mean and maximum M_{day} did not change with an increase of feeding ratio from 0.7 to 1.2 %BW (Table 6.3). However, mean and maximum M_F values were lower ($p < 0.05$) for fish fed at the largest feeding ration (Table 6.3). Calculated maximum M_F were between 33 and 46% larger than average rates.

6.3.5.- Apparent effect of fish stocking density on oxygen consumption

For fish weighing 11.8, 12.8, and 12.5 g the mean M_{day} and M_F increased with a decrease in stocking density from 316 to 107 %PCA (Table 6.3). Maximum M_{day} and M_F were similar for fish stocked at 316 and 212 %PCA, and both were lower than fish stocked at 107 %PCA (Table 6.3). Overall, observed maximum M_{day} and M_F were between 25 and 30% larger than average rates.

For fish weighing 113.3 and 113.8 g the mean M_{day} did not change with a decrease in stocking density from 275 to 189 %PCA (Table 6.3). Maximum M_{day} was larger for fish stocked at 275 %PCA than for those stocked at 189 %PCA (Table 6.3). Mean and maximum M_F for juvenile California halibut weighing 113.3 and 113.8 g, fed at 0.7 %BW, were not significantly different between the stocking densities tested ($p > 0.05$). Overall, calculated maximum M_F were between 41 and 54% larger than average rates.

For fish weighing 126.5 and 131.6 g the mean M_{day} and M_F did not change with a decrease in stocking density from 287 to 200% PCA, respectively (Table 6.3). Maximum M_{day} and M_F did not change either (Table 6.3). Overall, observed maximum M_{day} and M_F were between 37 and 46% larger than average rates.

For fish weighing 133.0 and 132.4 g the mean M_{day} and M_F did not change with a decrease in stocking density from 296 to 94% PCA, respectively (Table 6.3). Maximum M_{day} and M_F did not change either (Table 6.3). Overall, observed maximum M_{day} and M_F were between 33 and 41% larger than average rates.

6.3.6.- Diel pattern of oxygen consumption

Daily management at the experimental hatchery facilities included cleaning the raceways and loading the belt feeders at around 9:00 h. At this time DO probes were also cleaned. The oxygen consumption data obtained above gave a valuable insight into diel variations in hourly oxygen consumption rates.

Consumption of oxygen by juvenile California halibut with mean mass between 3.2 and 14.2 g, stocked at densities between 107 and 243% PCA, showed a clear diel change (Fig. 6.4 to Fig. 6.12). The ratios between maximum and mean oxygen consumption rates were between 1.20 and 1.30. Oxygen consumption rate increased sometime after 9:00 h, and continued increasing up to approximately 21:00 h. After 21:00 h the oxygen consumption rate decreased reaching a minimum around 7:00 h. This minimum sometimes was extended until 10:00 h. Lights went off and on abruptly, but no peaks in oxygen consumption rates were observed with this action, except for 12 g fish (Fig. 6.9 to Fig. 6.11). However, a peak was observed for almost all fish sizes between 3.2 and 14.2 g around 6:00 h, approximately 1 h before the lights were turned on; another peak was observed around 9:00 – 10:00 h, probably related with the DO probe and raceway cleaning procedures. No peaks were observed around the time when the lights were turned off. Oxygen consumption rates seemed to be linked to the feed distribution, which started around 9:30 h and ended around 21:30 h. Maximum oxygen consumption during the light period was observed sometime after 18:00 h and it remained elevated until 22:00 h.

Fish groups weighing 3.2, 4.4, and 6.2 g were reared at a similar %PCA and feed ration (Table 6.3). The mean M_{day} for those groups decreased with increasing fish size (Table 6.3). However, maximum M_{day}, and mean and maximum M_F were not significantly different with varying fish size. The ratios of maximum to mean oxygen consumption for fish weighing 3.2, 4.4, and 6.2 g were 1.21, 1.24, and 1.25, respectively.

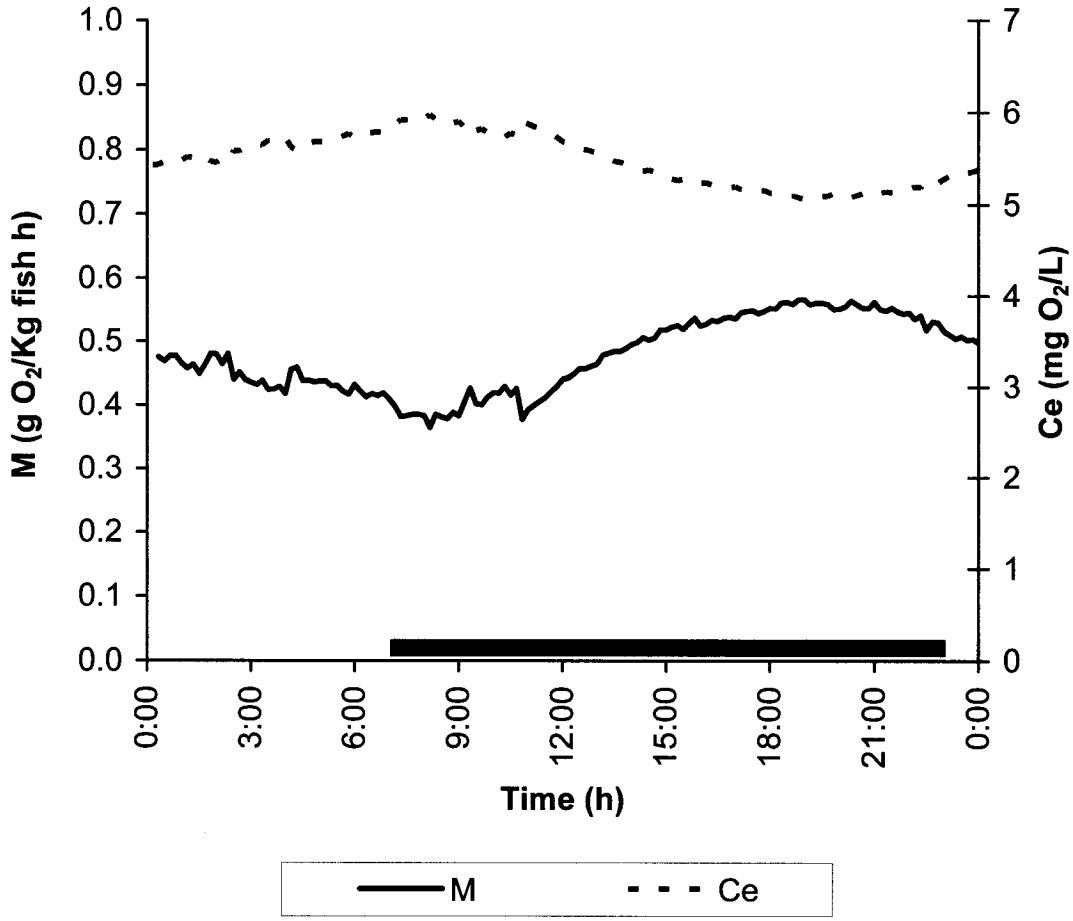

Figure 6.4.- Diel pattern of oxygen consumption rates (M) for 3.2 g juvenile California halibut reared at a density of 209% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.07 and Ce±0.29). The black bar indicates the time when lights were on.

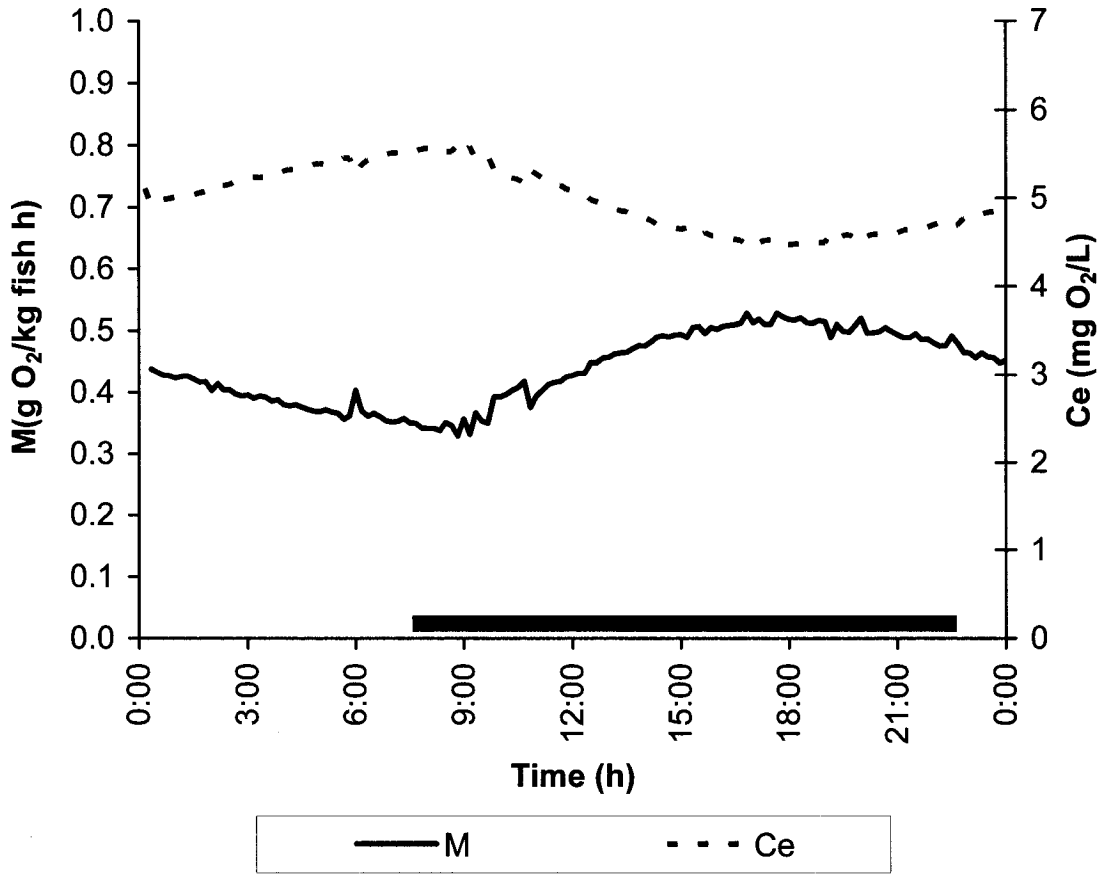

Figure 6.5.- Diel pattern of oxygen consumption rates (M) for 4.4 g juvenile California halibut reared at a density of 279% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.05 and Ce±0.33). The black bar indicates the time when lights were on.

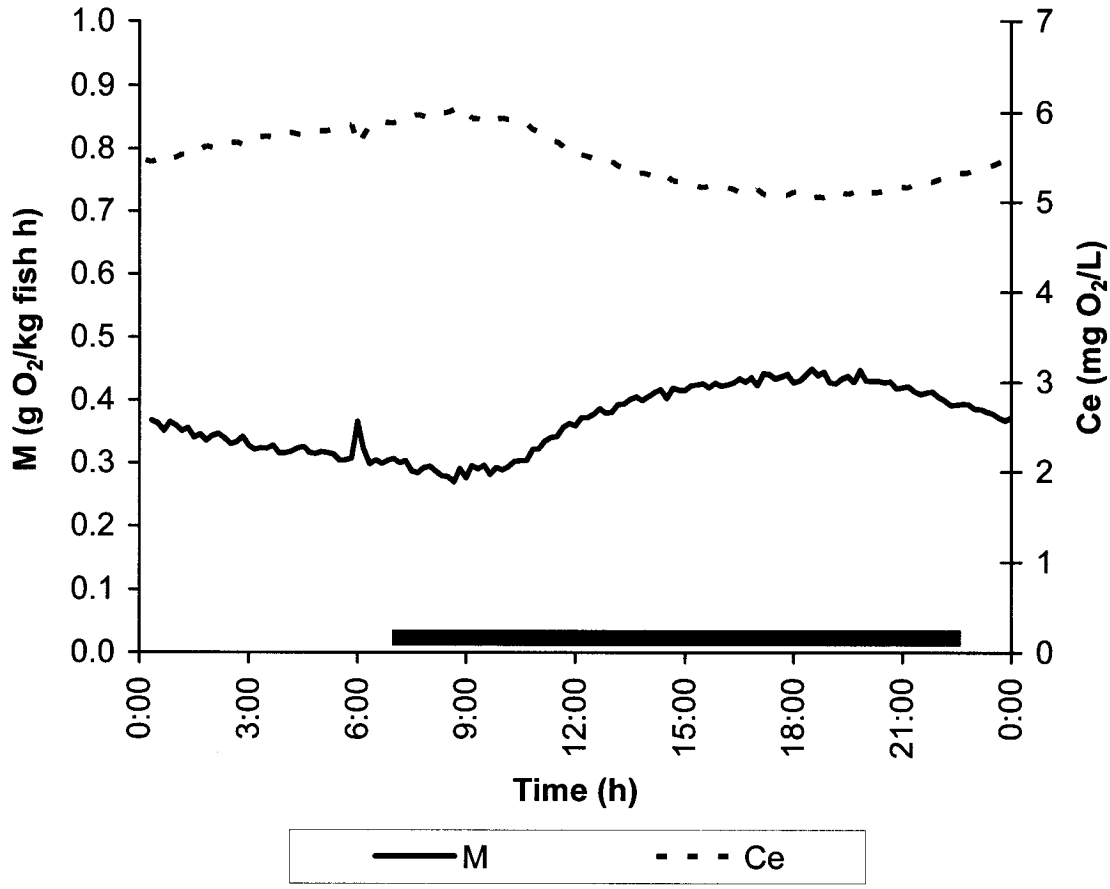

Figure 6.6.- Diel pattern of oxygen consumption rates (M) for 6.2 g juvenile California halibut reared at a density of 179% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.06 and Ce±0.22). The black bar indicates the time when lights were on.

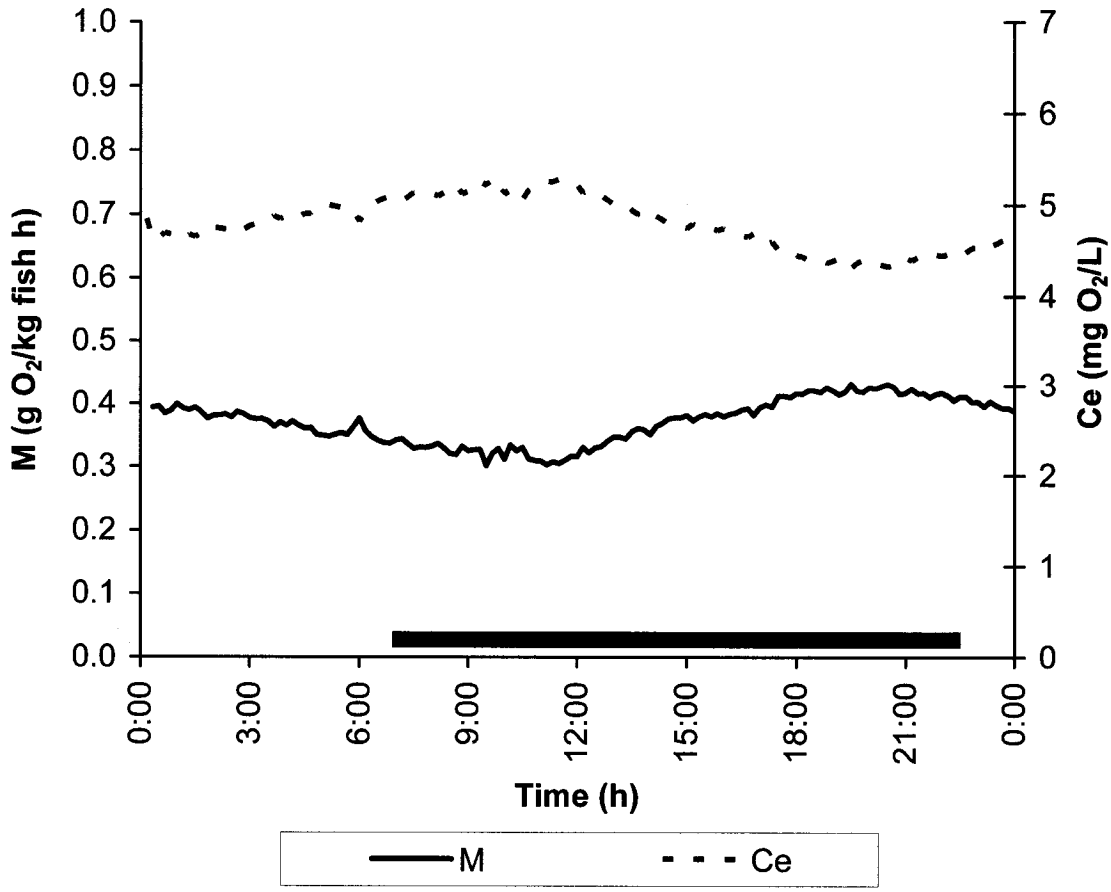

Figure 6.7.- Diel pattern of oxygen consumption rates (M) for 7.5 g juvenile California halibut reared at a density of 351% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.05 and Ce±0.55). The black bar indicates the time when lights were on.

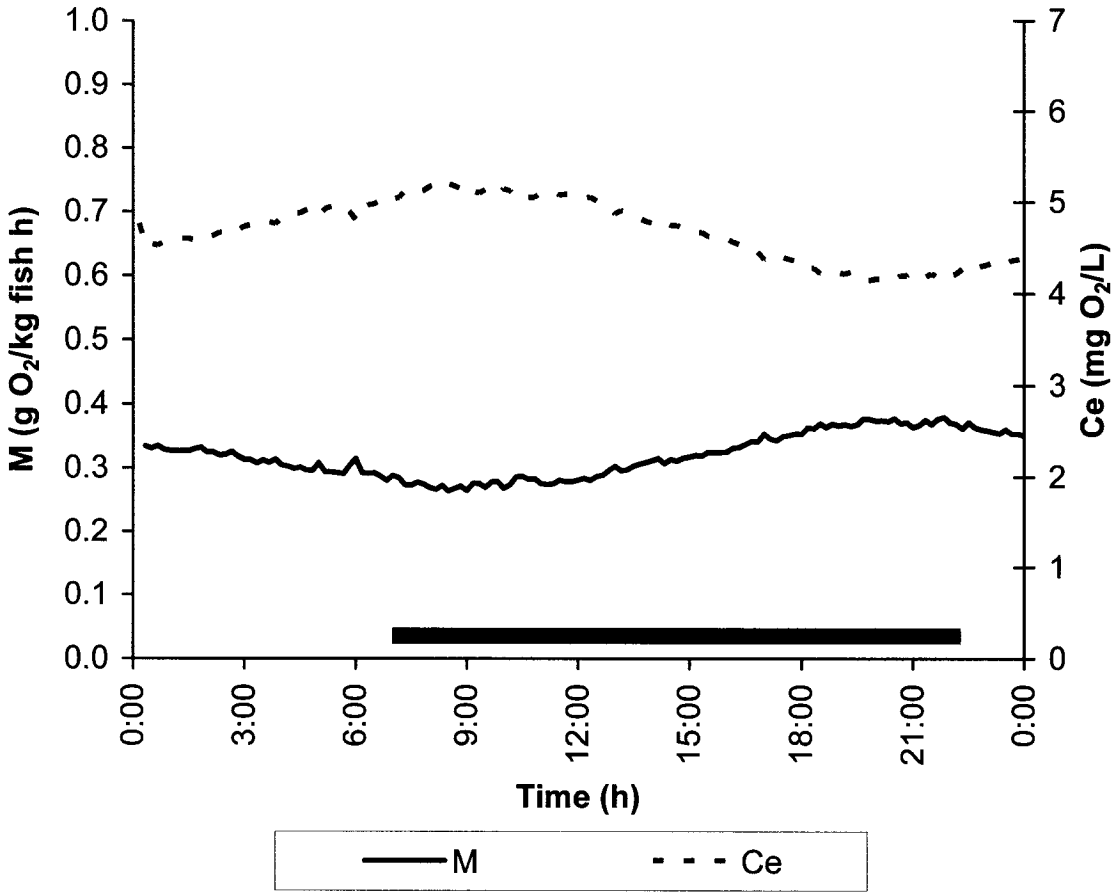

Figure 6.8.- Diel pattern of oxygen consumption rates (M) for 9.2 g juvenile California halibut reared at a density of 389% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.06 and Ce±0.63). The black bar indicates the time when lights were on.

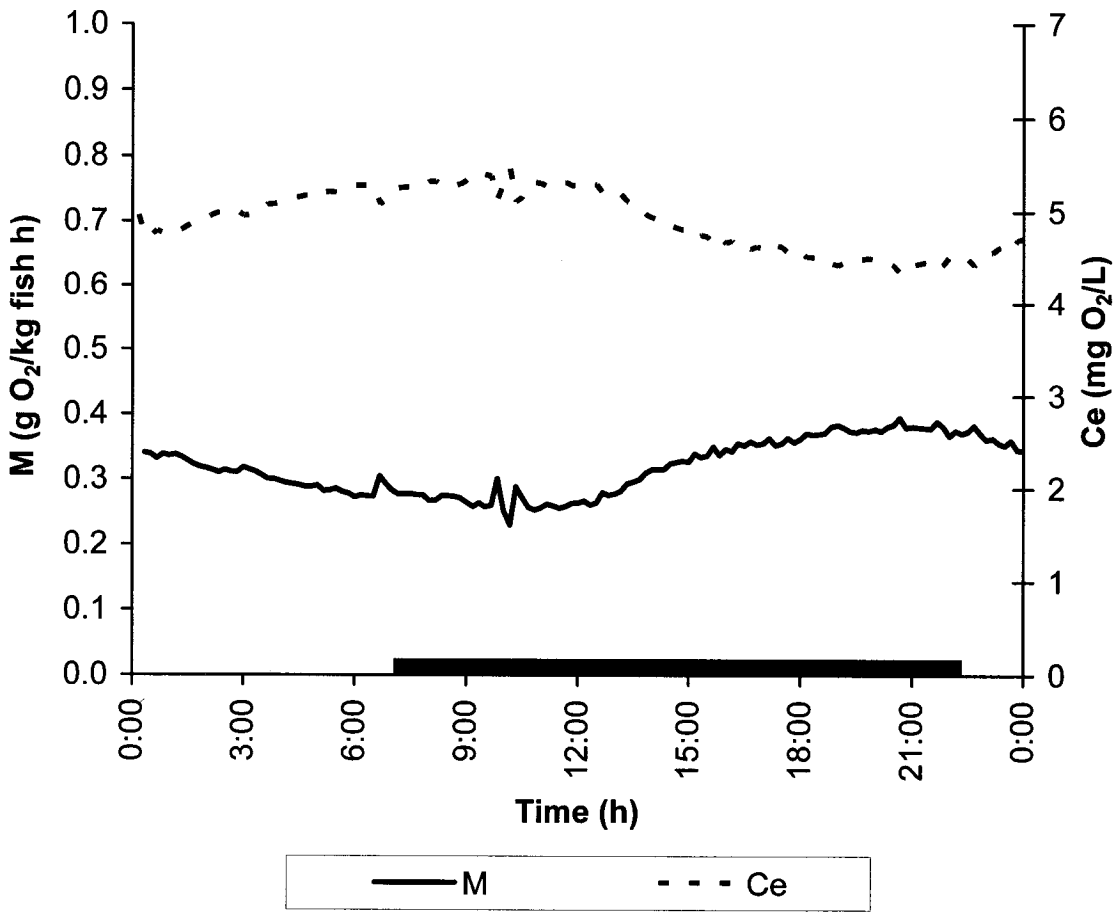

Figure 6.9.- Diel pattern of oxygen consumption rates (M) for 11.8 g juvenile California halibut reared at a density of 316% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.06 and Ce±0.50). The black bar indicates the time when lights were on.

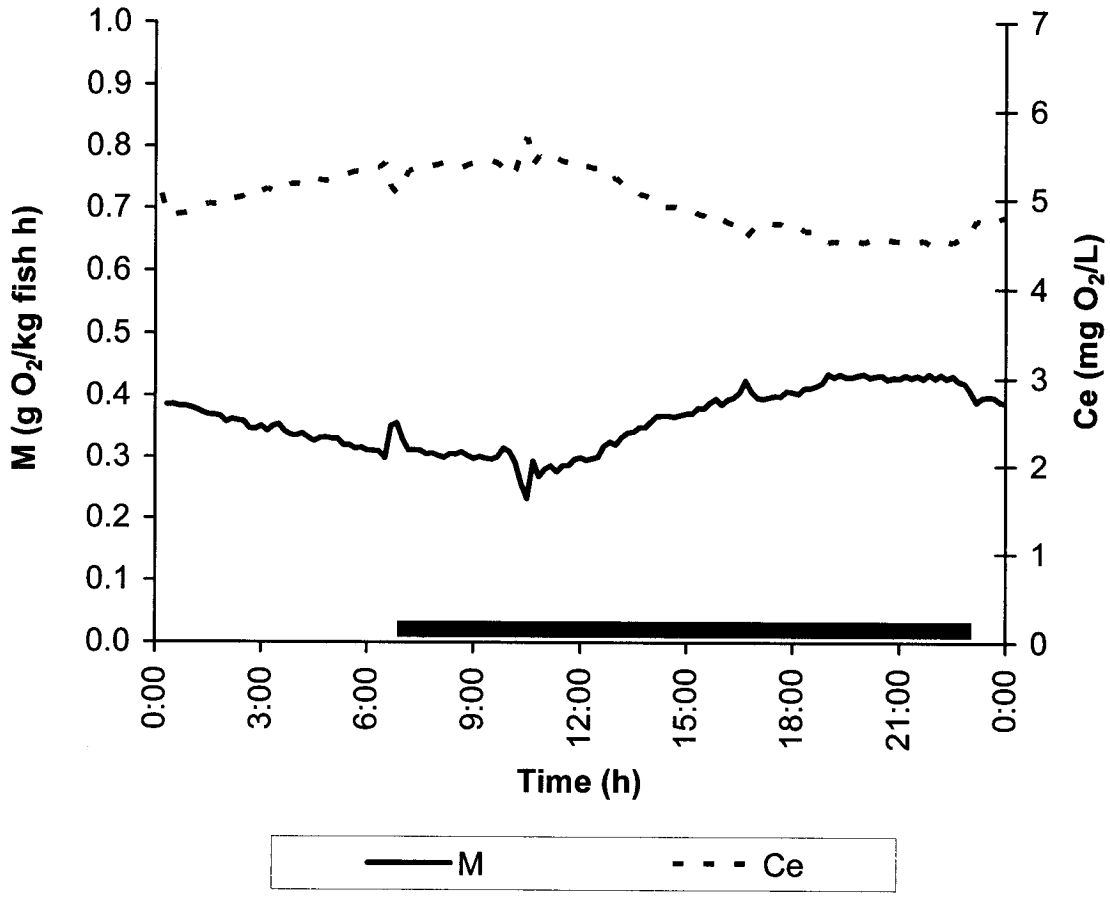

Figure 6.10.- Diel pattern of oxygen consumption rates for 12.8 g juvenile California halibut reared at a density of 212% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.15 and Ce±0.83). The black bar indicates the time when lights were on.

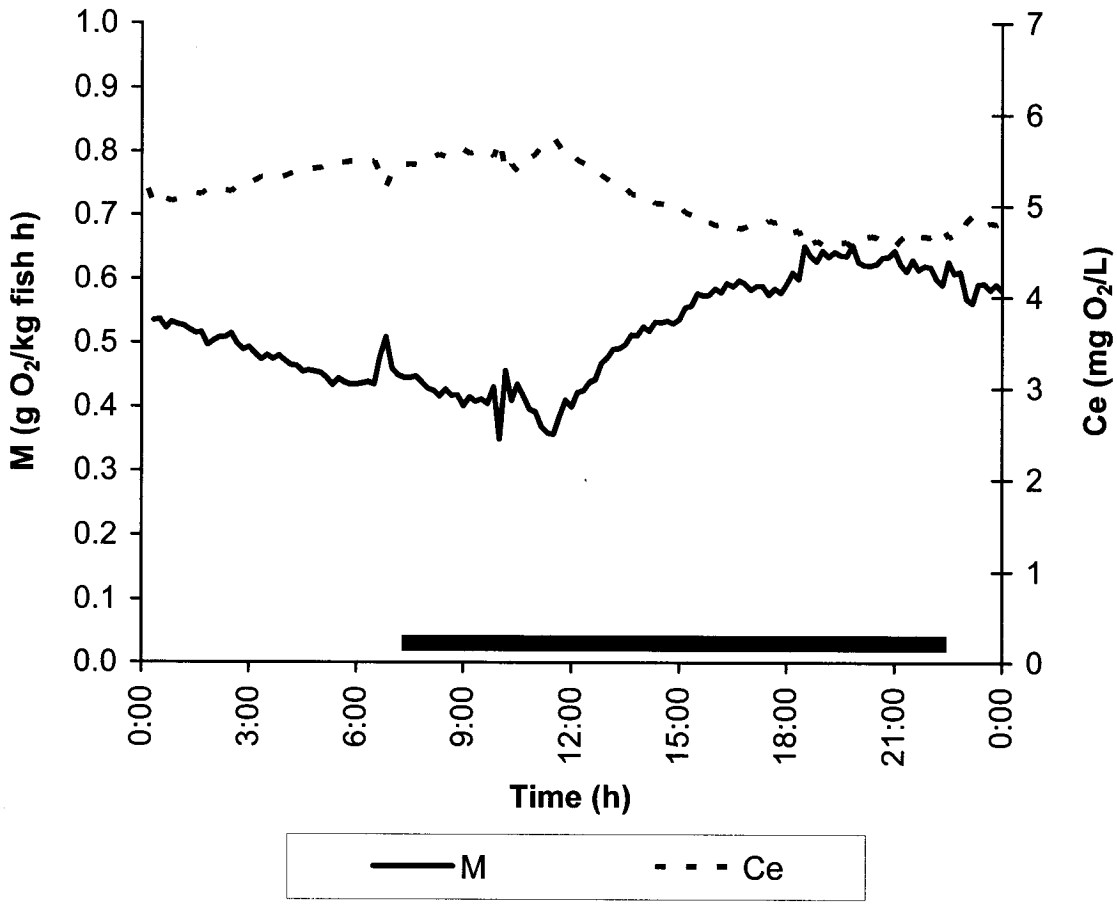

Figure 6.11.- Diel pattern of oxygen consumption rates for 12.5 g juvenile California halibut reared at a density of 107% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.10 and Ce±0.48). The black bar indicates the time when lights were on.

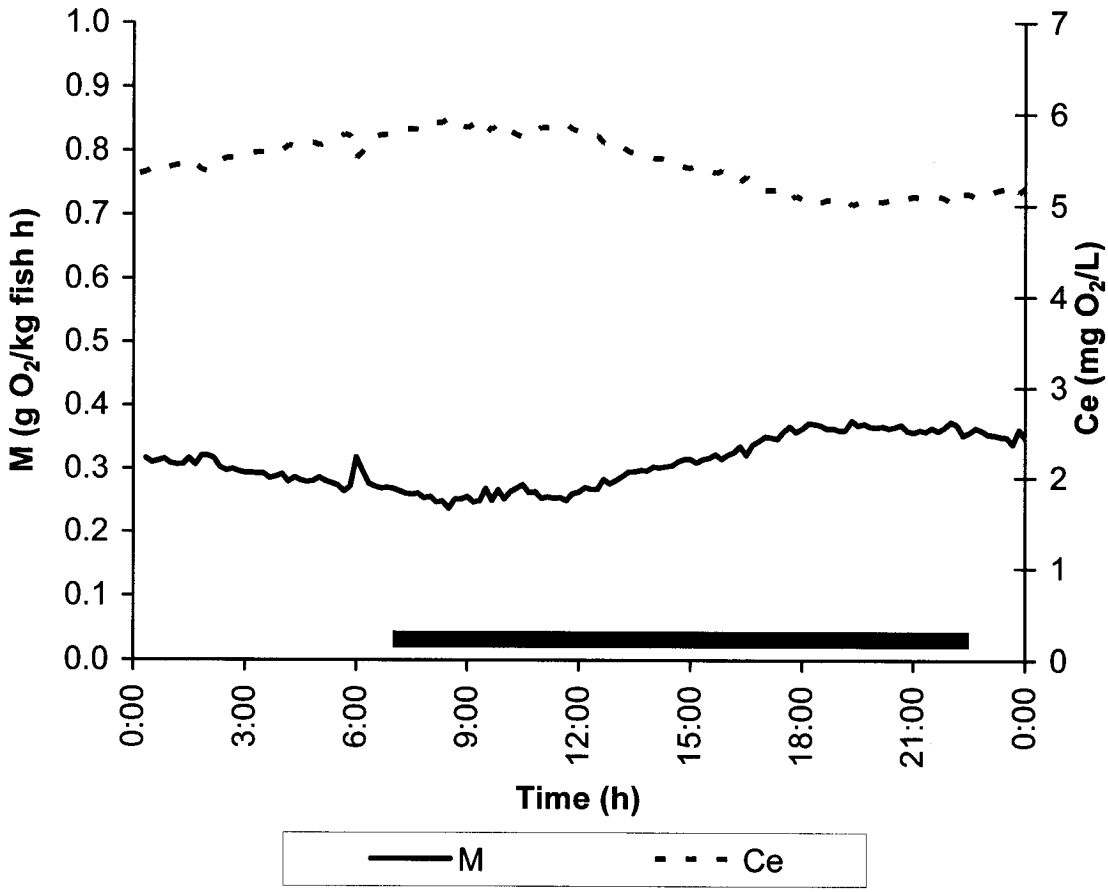

Figure 6.12.- Diel pattern of oxygen consumption rates for 14.2 g juvenile California halibut reared at a density of 243% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.05 and Ce±0.35). The black bar indicates the time when lights were on.

Fish groups weighing 7.5 to 9.2 were reared at a similar %PCA and feed ration (Table 6.3). The mean and maximum M_{day} for those groups decreased with increasing fish size (Table 6.3); however, mean and maximum M_F were not significantly different with varying fish size. The ratio of maximum to mean oxygen consumption for fish weighing 7.5 and 9.2 g were 1.20 and 1.22, respectively.

Fish groups weighing 11.8, 12.8, and 12.5 g were fed with a similar ration of 1.2 %BW and stocked at densities of 316, 212, and 107 %PCA, respectively (Table 6.3). The mean M_{day} and M_F for those groups were significantly different ($p < 0.05$) for the three stocking densities. The maximum M_{day} and M_F were similar for the two largest densities, which were smaller than the one at 107 %PCA ($p < 0.05$) (Table 6.3). The ratio of maximum to mean oxygen consumption for fish weighing 11.8, 12, 8, and 12.5 g were 1.27, 1.25, and 1.30, respectively. Diel curves had the same shape for the three stocking densities (Fig. 6.9 to 6.11), but hourly oxygen consumption was larger for the fish stocked at 107 %PCA (Fig. 6.12).

The diel oxygen consumption for the largest fish (110.5 to 165.6 g) was different from the ones recorded for fish smaller than 14 g. The diel curves for the largest fish show several smaller peaks throughout the day, probably due to the excitability of these fish (Fig. 6.13 to Fig. 6.25).

Consumption of oxygen by juvenile California halibut with mean mass between 110.5 and 165.6 g, stocked at densities between 94 and 296% PCA, showed a clear diel change (Fig. 6.13 to Fig. 6.25). The ratio of maximum to mean oxygen consumption rates was between 1.29 and 1.54. Although the maximum to mean ratio was larger than those described above for California halibut under 14.2 g, the standard deviation for the hourly M_{day} was smaller for the largest fish. In general, the oxygen consumption rate started to increase sometime after 9:00 h, and continued increasing until approximately 21:00 h. Maximum hourly oxygen consumption during the light period was observed sometime between 18:00 h and 22:00 h. After 21:00 h the oxygen consumption rate decreased reaching a minimum around 7:00 h. Lights went off and on abruptly, but no peaks in oxygen consumption rates were observed with this action. Oxygen consumption rate did not change significantly after feed was distributed in contrast to California halibut weighing less than 14.2 g.

Fish groups weighing between 110.5 and 115.7 g were reared between 189 and 281% PCA, and fed at 0.7 % BW (Table 6.3). The mean M_{day} and the mean and maximum $M_{F\text{-}day}$ were not significantly different ($p > 0.05$). However, maximum M_{day} was larger for 113.3 g fish reared at 275% PCA (Table 6.3). The ratio maximum to mean oxygen consumption for fish weighing between 110.5 and 115.7 g were between 1.4 and 1.5. Diel curves had about same shape for the above stocking densities (Fig. 6.13 to 6.16). Overall, stock density effect on oxygen consumption, if there is one, is very small.

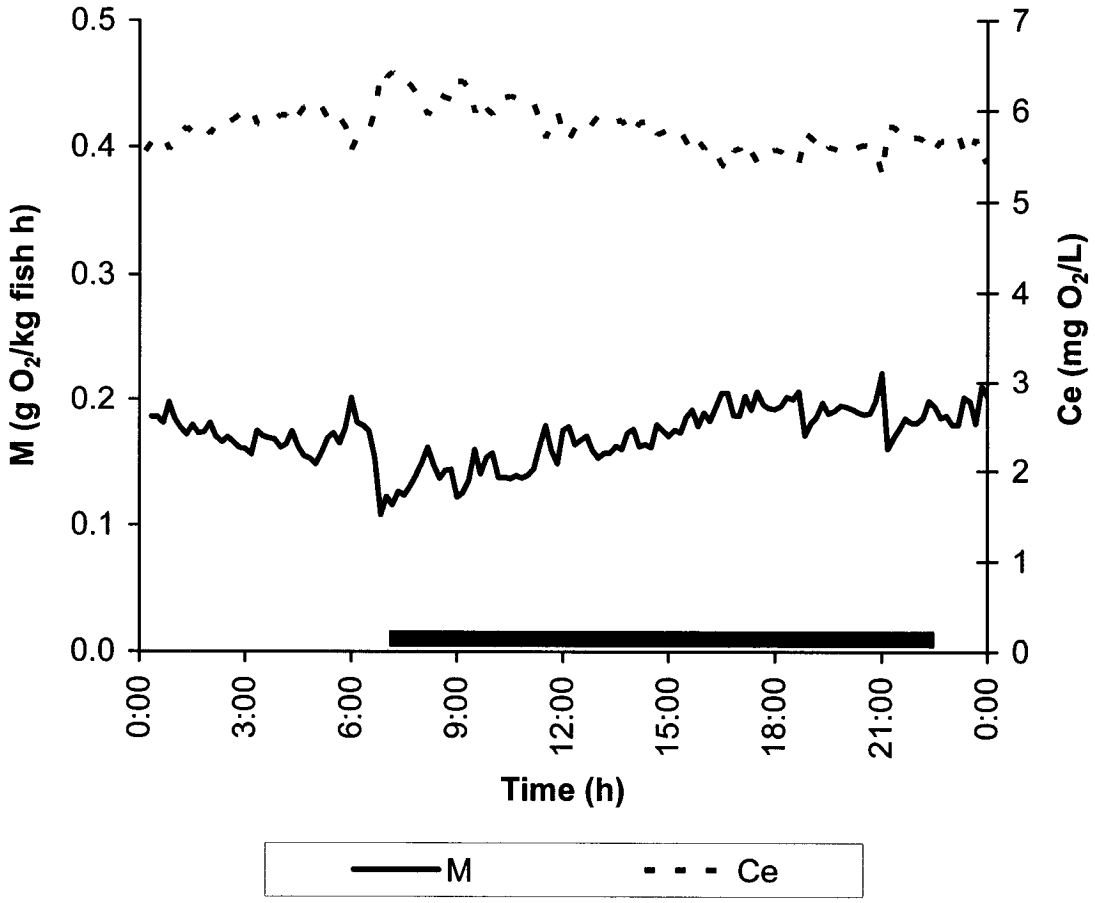

Figure 6.13.- Diel pattern of oxygen consumption rates for 110.5 g juvenile California halibut reared at a density of 270% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.11 and Ce±1.03). The black bar indicates the time when lights were on.

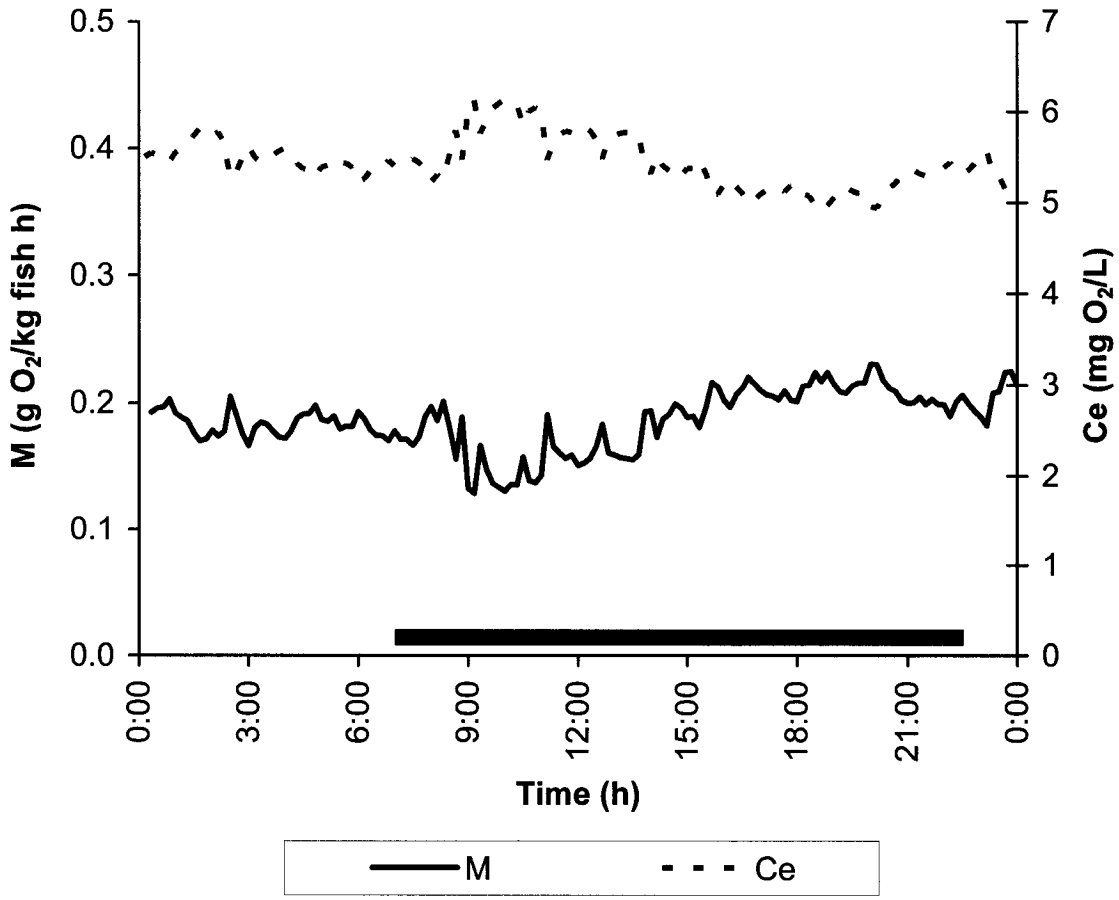

Figure 6.14.- Diel pattern of oxygen consumption rates for 113.3 g juvenile California halibut reared at a density of 275% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.10 and Ce±1.22). The black bar indicates the time when lights were on.

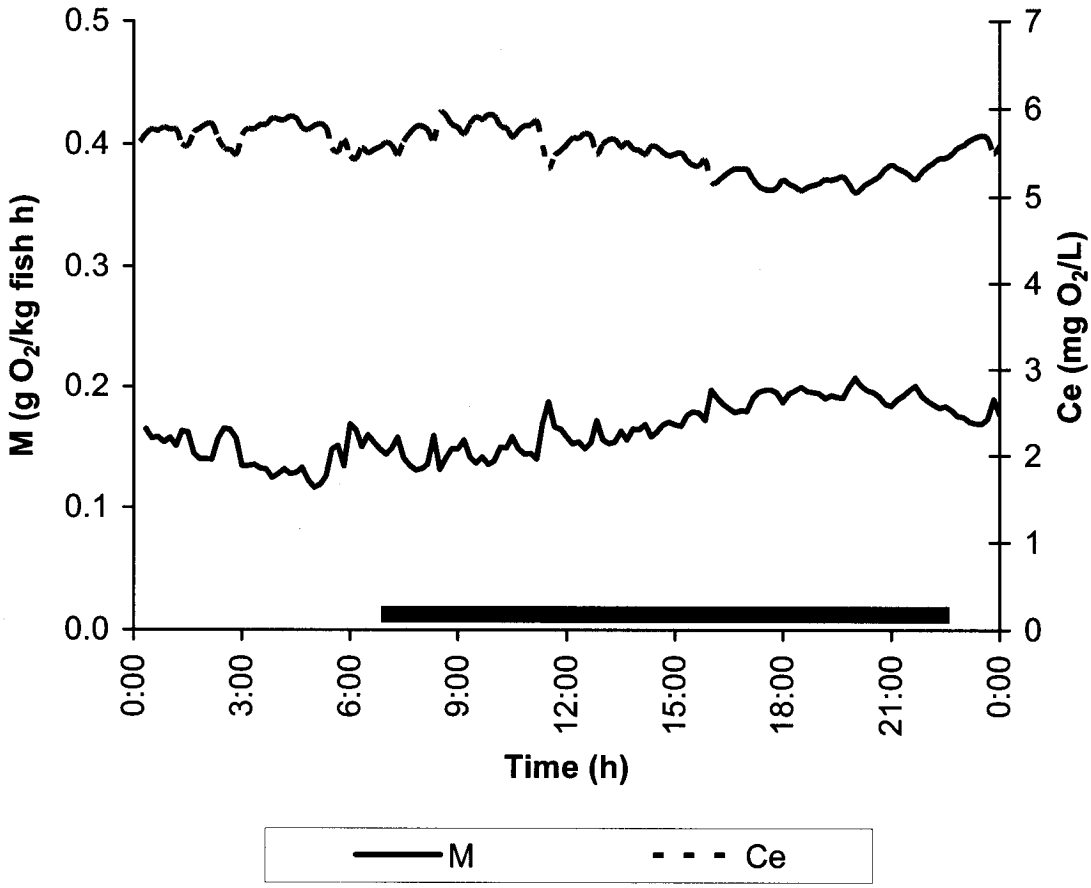

Figure 6.15.- Diel pattern of oxygen consumption rates for 113.8 g juvenile California halibut reared at a density of 189% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.08 and Ce±0.68). The black bar indicates the time when lights were on.

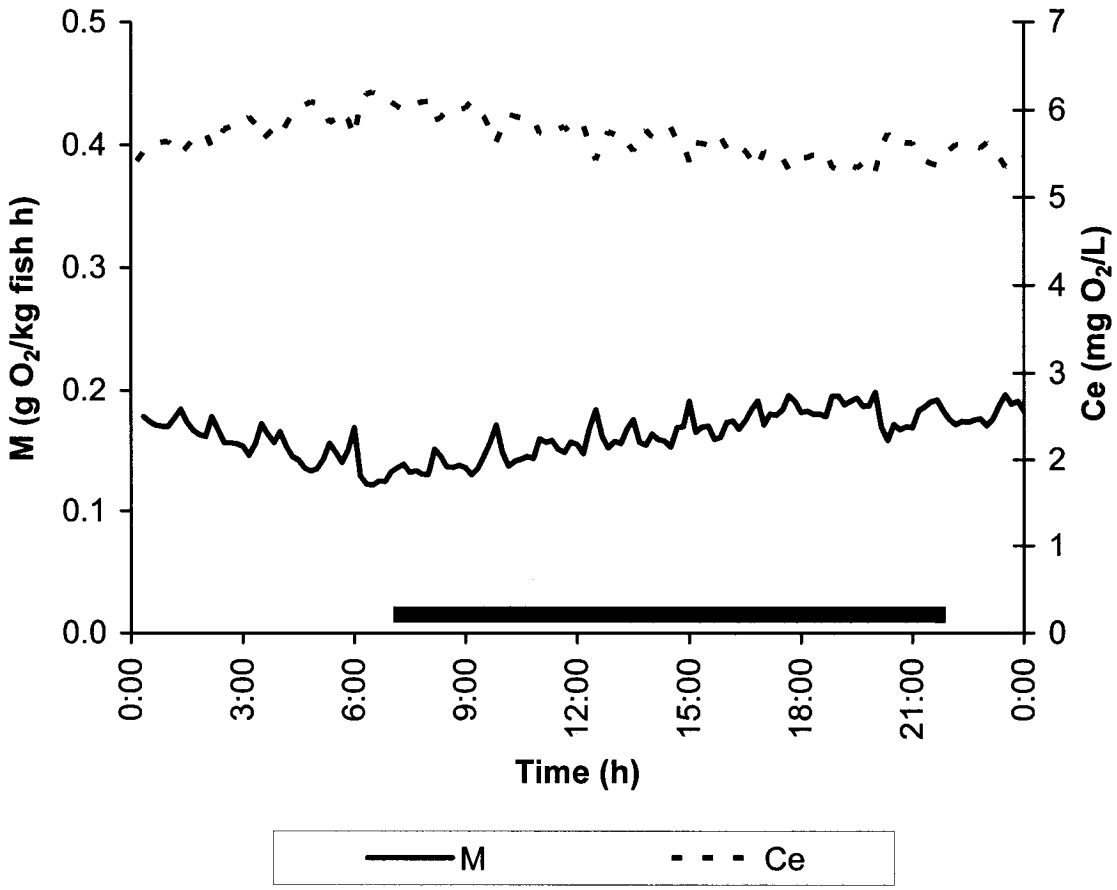

Figure 6.16.- Diel pattern of oxygen consumption rates for 115.7 g juvenile California halibut reared at a density of 281% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.11 and Ce±1.24). The black bar indicates the time when lights were on.

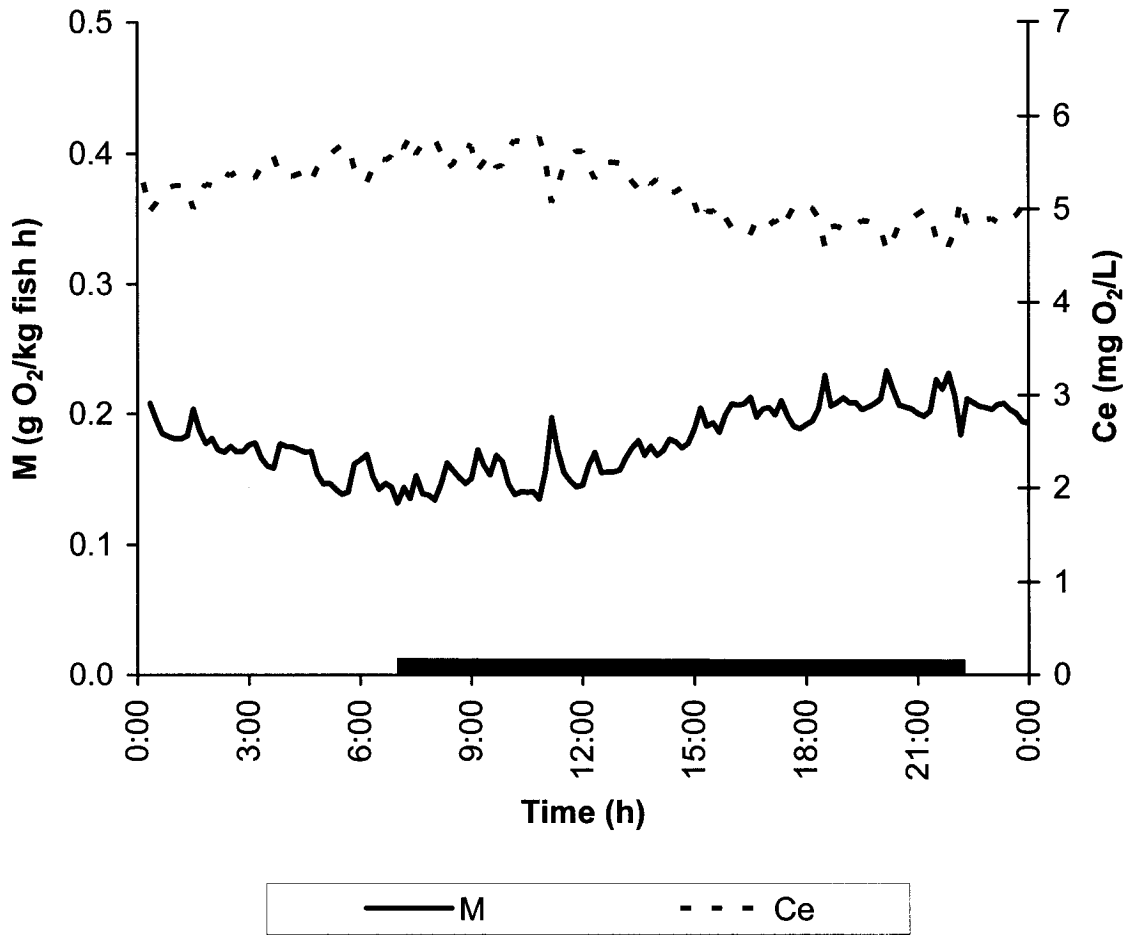

Figure 6.17.- Diel pattern of oxygen consumption rates for 117.9 g juvenile California halibut reared at a density of 281% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.08 and Ce±0.83). The black bar indicates the time when lights were on.

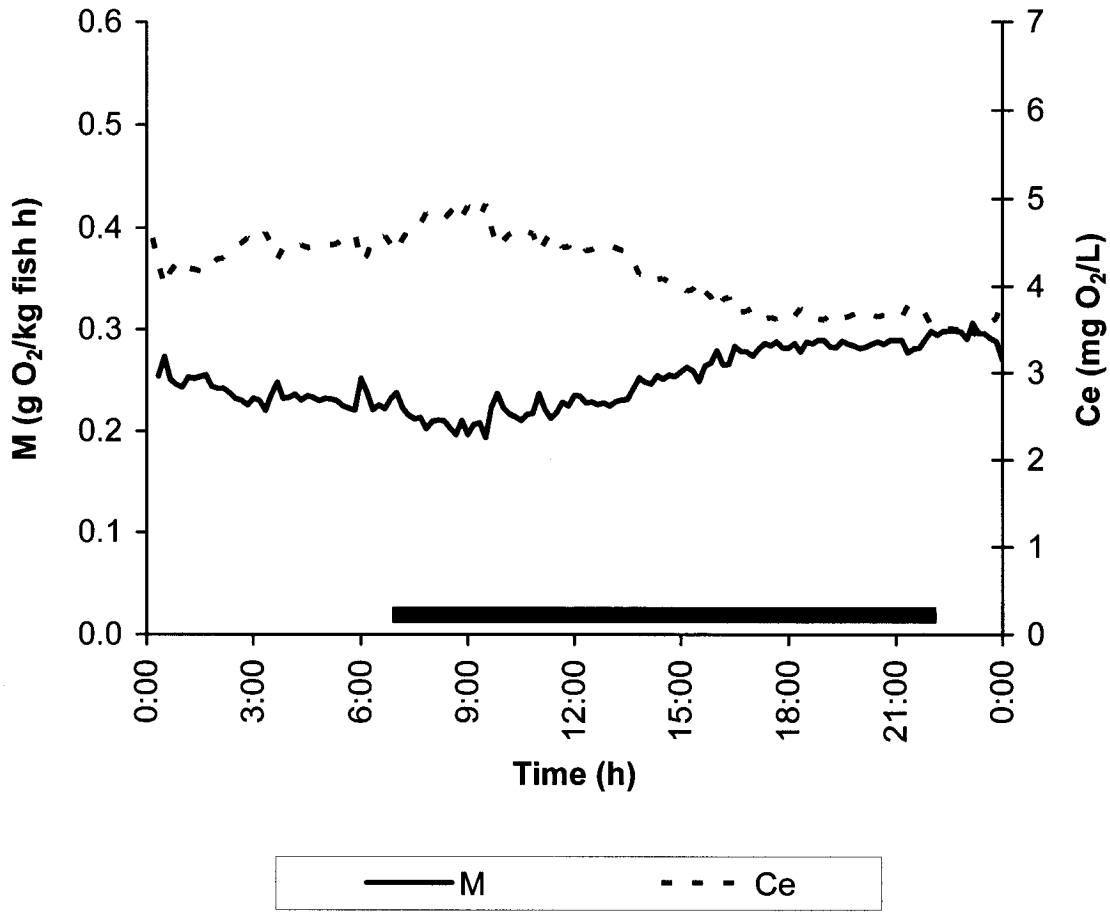

Figure 6.18.- Diel pattern of oxygen consumption rates for 120.1 g juvenile California halibut reared at a density of 195% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.08 and Ce±1.03). The black bar indicates the time when lights were on.

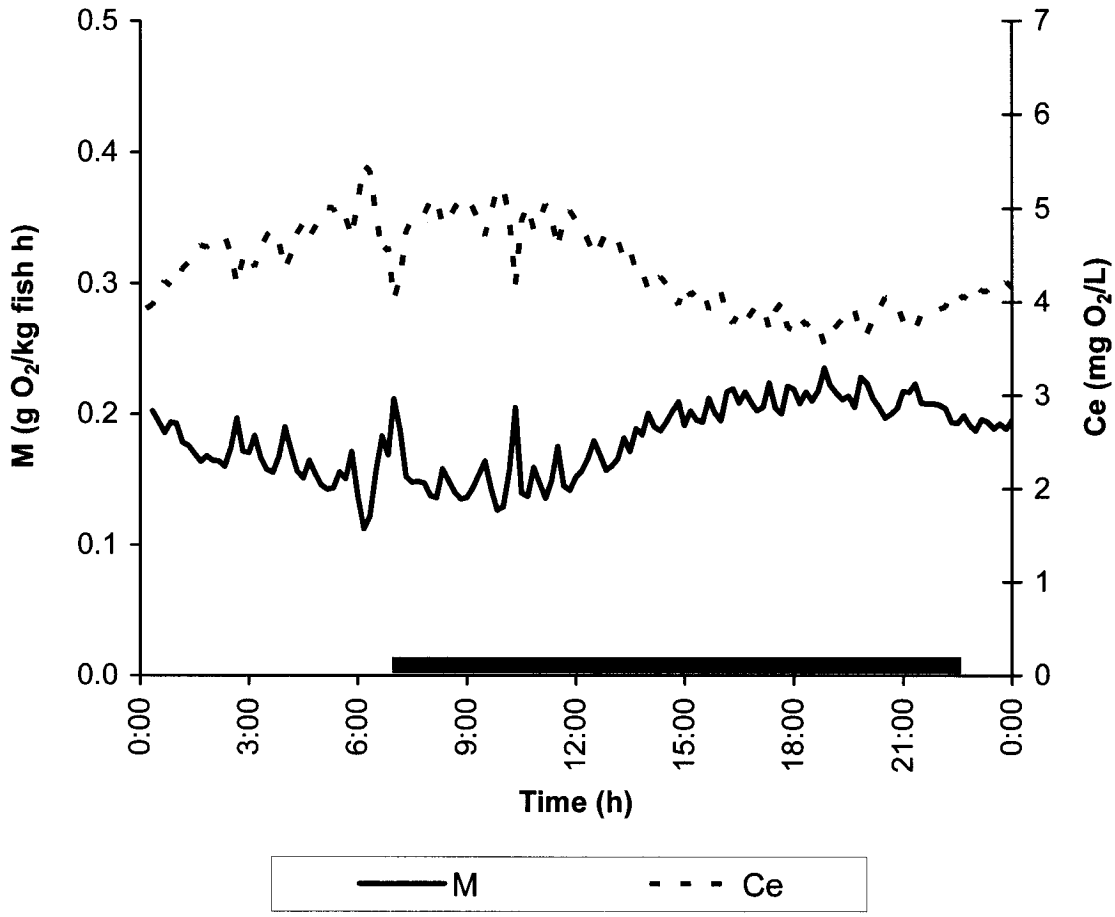

Figure 6.19.- Diel pattern of oxygen consumption rates for 126.5 g juvenile California halibut reared at a density of 287% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.08 and Ce±0.94). The black bar indicates the time when lights were on.

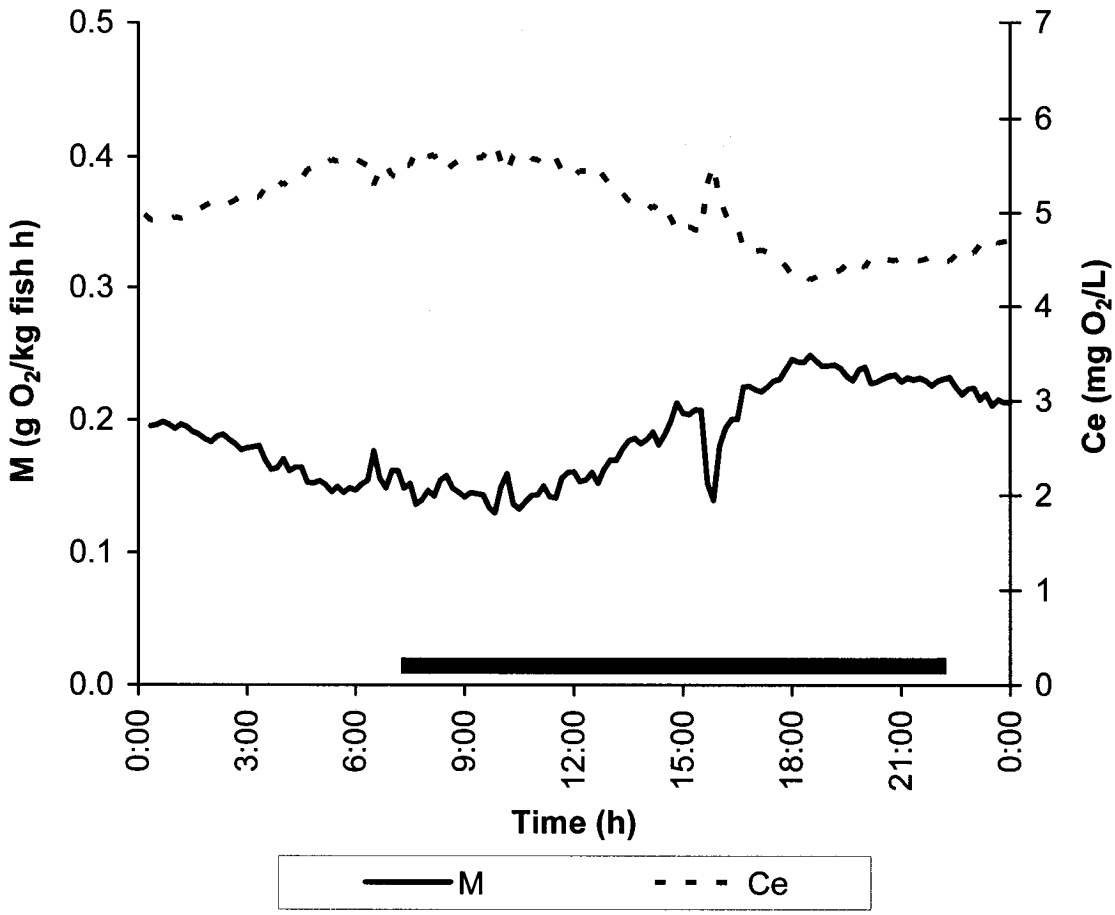

Figure 6.20.- Diel pattern of oxygen consumption rates for 131.6 g juvenile California halibut reared at a density of 200% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.09 and Ce±0.94). The black bar indicates the time when lights were on.

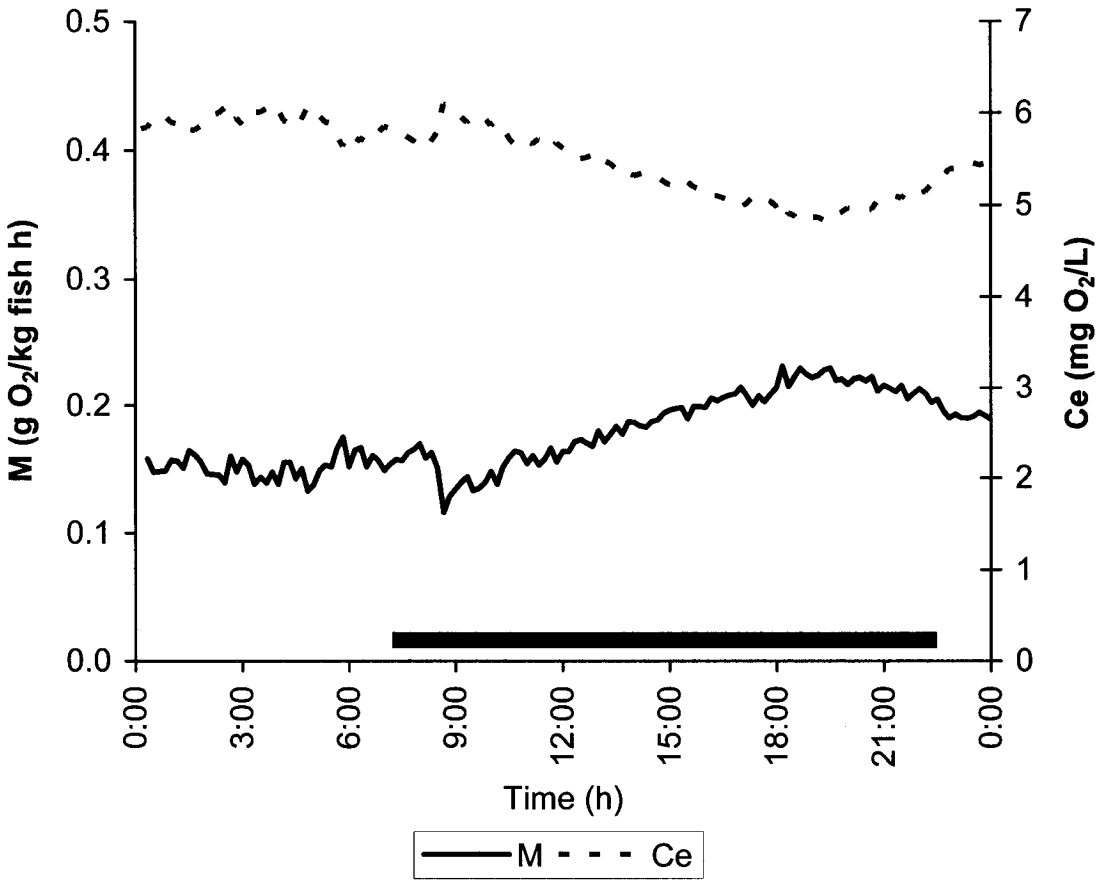

Figure 6.21.- Diel pattern of oxygen consumption rates for 132.4 g juvenile California halibut reared at a density of 94% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.07 and Ce±0.78). The black bar indicates the time when lights were on.

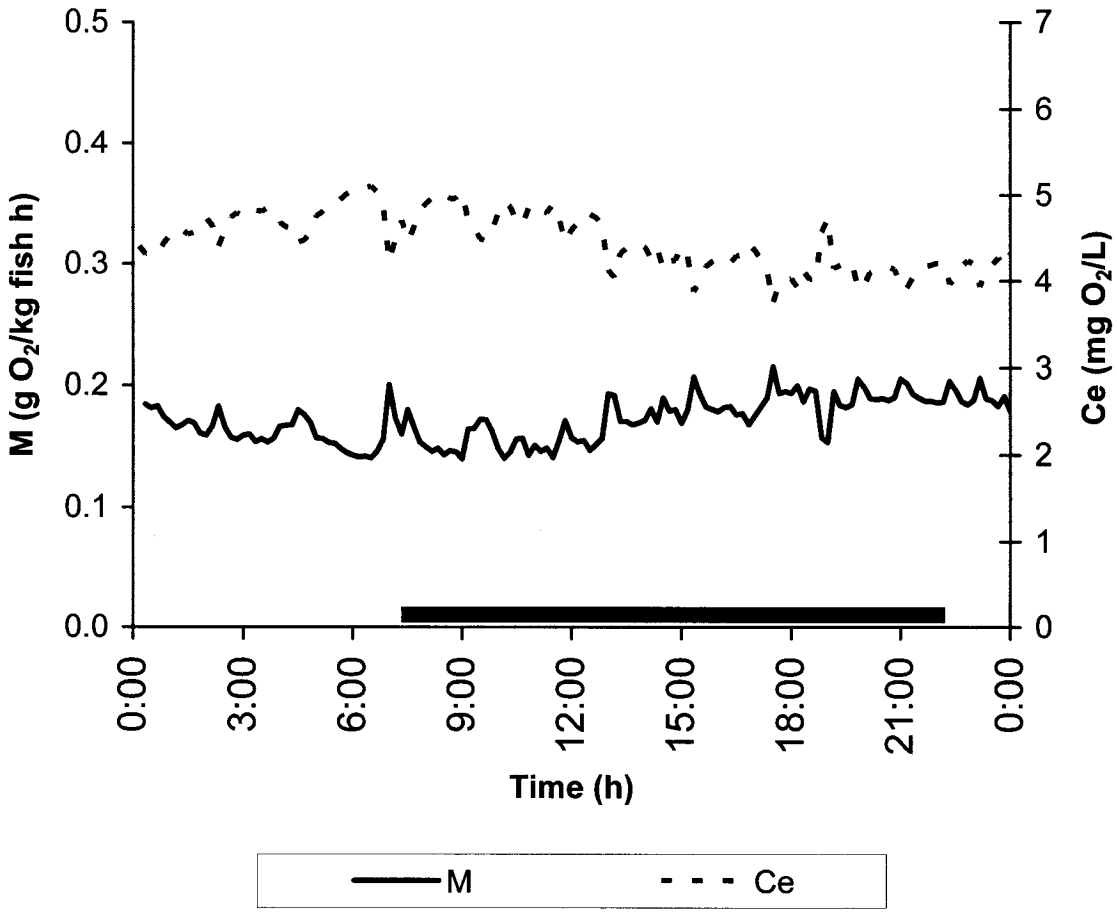

Figure 6.22.- Diel pattern of oxygen consumption rates for 133.0 g juvenile California halibut reared at a density of 296% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.06 and Ce±1.04). The black bar indicates the time when lights were on.

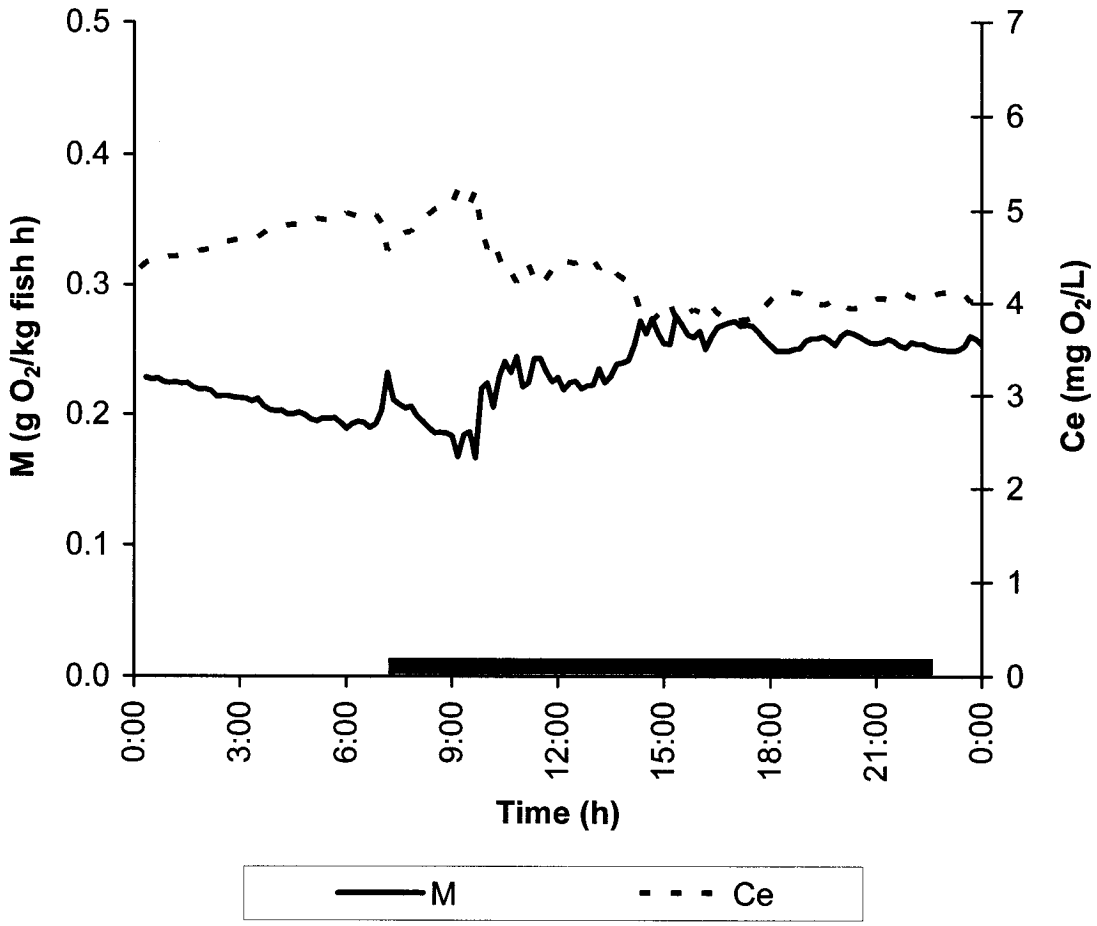

Figure 6.23.- Diel pattern of oxygen consumption rates for 140.6 g juvenile California halibut reared at a density of 98% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.06 and Ce±0.78). The black bar indicates the time when lights were on.

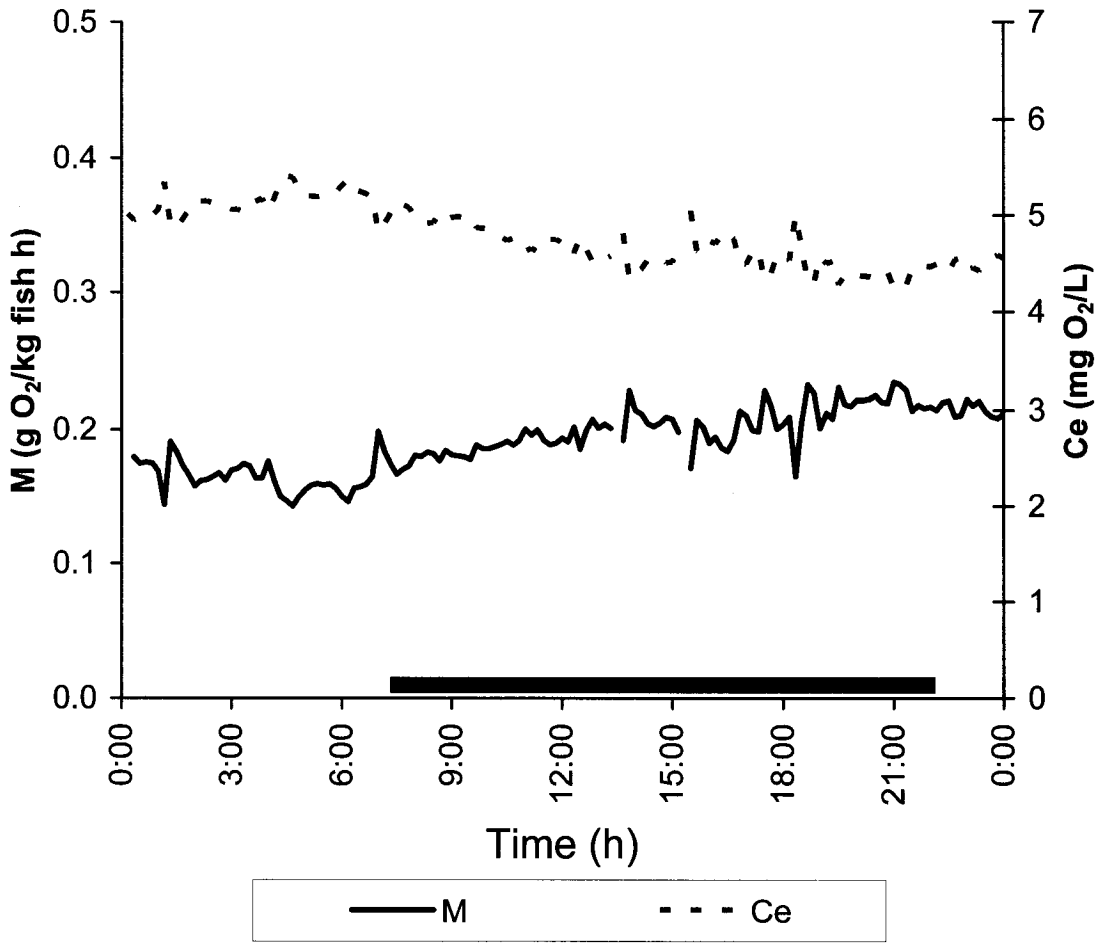

Figure 6.24.- Diel pattern of oxygen consumption rates for 141.6 g juvenile California halibut reared at a density of 208% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.10 and Ce±1.23). The black bar indicates the time when lights were on.

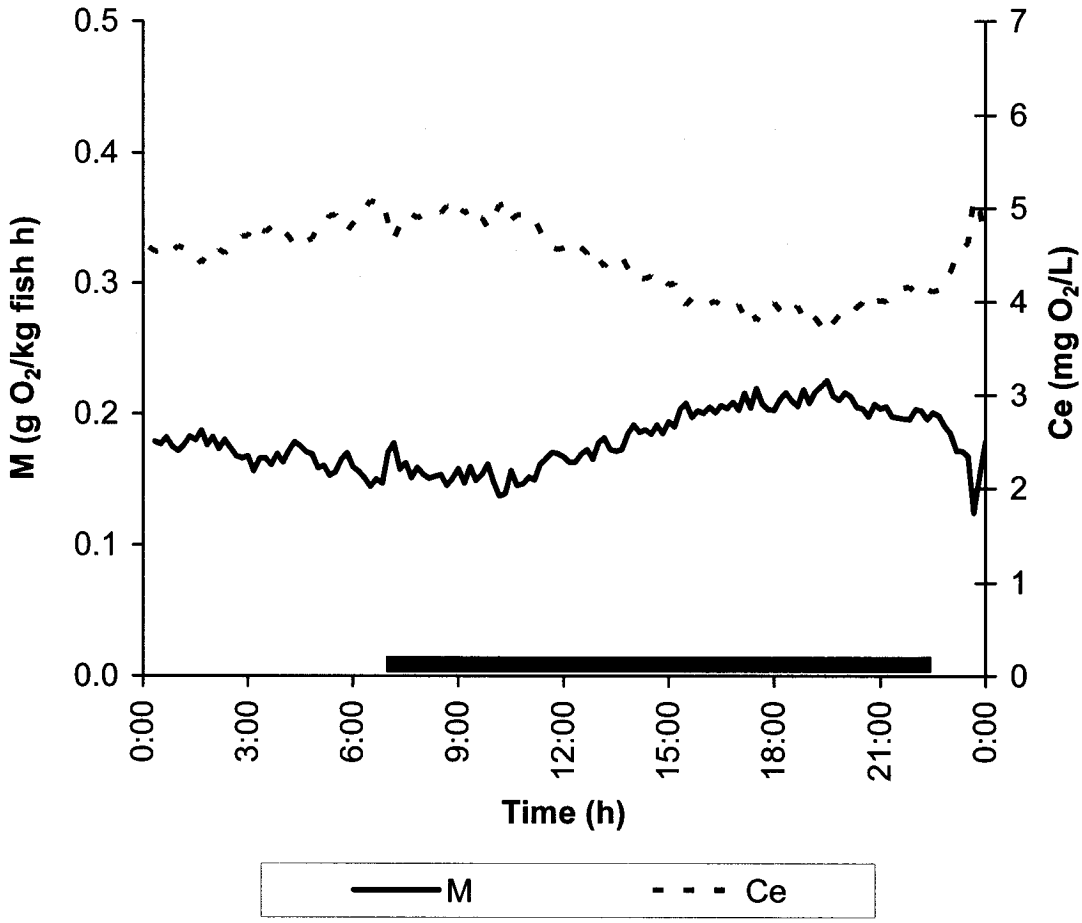

Figure 6.25.- Diel pattern of oxygen consumption rates for 165.6 g juvenile California halibut reared at a density of 106% PCA. Ce stands for dissolved oxygen concentration in the effluent. Mean values of three consecutive days are plotted (Maximum standard deviation M±0.08 and Ce±1.17). The black bar indicates the time when lights were on.

In general, fish groups weighing between 110.5 and 165.6 g were fed between 0.7 and 1.2 %BW and reared between 94 and 296% PCA (Table 6.3). Overall, the mean and maximum M_{day} for those fish were not significantly different ($p > 0.05$) (Table 6.3). However, mean and maximum M_F were significantly different and larger for fish fed at 0.7 than for 1.2 % BW, with a few exceptions. The ratio of maximum to mean oxygen consumption for fish weighing between 110.5 and 165.6 g were between 1.29 and 1.54, which were larger than those for fish weighing between 3.2 and 14.2 g.

6.3.7.- Oxygen consumption rate and fish size relationship

With the results described above a relationship between average body mass and oxygen consumption over a 24 hour period was obtained and plotted for all combinations of feed rations, stocking densities and fish biomass (Fig. 6.26). Overall, the daily average and maximum rate of oxygen consumption per unit body mass decreased with increasing body mass for juvenile California halibut between 3.2 and 165.6 g biomass reared at 21.56 ± 0.97 °C under farm-like conditions. The relationship between W and average M_{day} was:

$$M_{day} = 15.077 * W^{-0.2452} \quad ,r^2 = 0.83 \qquad \text{(Eq. 6.16)}$$

And the relationship between W and maximum M_{day} was:

$$M_{day} = 17.266 * W^{-0.2033} \quad ,r^2 = 0.79 \qquad \text{(Eq. 6.17)}$$

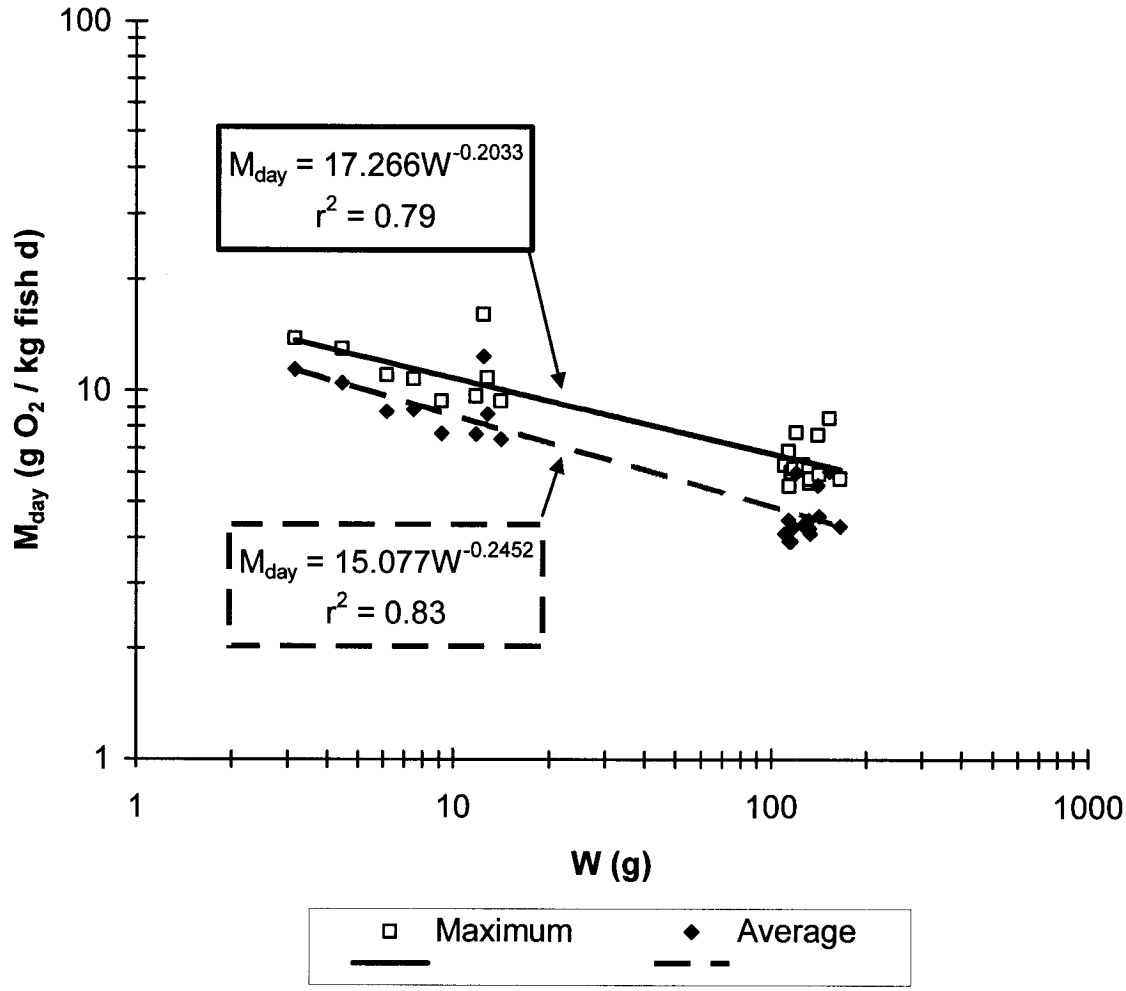

Figure 6.26.- Relationship between average and maximum daily oxygen consumption rate (M_{day}) and mean body mass (W) for juvenile California halibut. Fish were between 3.2 and 165.6 g mass. Fish were stocked between 94 and 389% PCA. Fish were fed between 3.0 and 0.7 % BW.

6.4.- DISCUSSION

A comprehensive knowledge of the oxygen consumption of California halibut is of vital importance for the design of the system for water and oxygen supply in a recirculating culture facility. Although the methods used by several authors for determining oxygen consumption rates have some basic differences, the general consensus is that the oxygen uptake is directly proportional to the water temperature and feeding ration, and inversely proportional to fish size (Brown, 1980; Forsberg, 1994; Tudor, 1999). California halibut tested were reared in a recirculating system, with water quality parameters set to optimize growth and survival. For the present research, juvenile California halibut were reared for several months at 21.6 ± 1.0 °C, and many open respirometries were performed under farm-like conditions.

6.4.1.- Water quality

In this six month experiment, salinity varied between 27.5 and 32.5 g/L and temperature between 19.2 and 23.18 °C. California halibut studies on the effects of temperature and salinity on growth have been reported by Innis (1980) and Madon (2002). Salinity and temperature were within the recommended values given by Innis (1980) and Madon (2002) for culture of California halibut juveniles.

The TAN and nitrite-N levels remained below 1 mg/L throughout the study. No studies on TAN and nitrite toxicity have been published for California halibut.

Person Le-Ruyet et al. (1997) reported that a TAN concentration up to 13 mg TAN/L (34.5 g/L salinity; 17 °C; pH 7.9; 6 mg/L dissolved oxygen) had no effect on survival and growth rate of turbot weighing between 13 and 104 g. There is no literature regarding toxicity of nitrite for flatfish species. However, recommended levels for mariculture practices of 0.5 mg NO_2-N/L have been suggested (Blancheton, 2000; Blanchard et al., 2003). In general TAN and nitrite-N values observed in this study were lower than those concentrations reported in the literature as growth limiting.

6.4.2.- Oxygen drop in an unstocked raceway

Raceway surfaces in contact with the water were cleaned every morning by scrubbing although this activity is unlikely to be practical for a commercial facility. It was observed in the present research that almost no fouling grew in raceways stocked with juvenile California halibut over 150% PCA. Tudor (1993) reported both production and consumption of oxygen by fouling organisms to have a day-night cycle. In the present study, an unstocked biofouled raceway was monitored for several days, and no day-night change in dissolved oxygen consumption was observed. The oxygen drop in the unstocked raceway was 1.30±0.53% (% of influent concentration), which was about 18.4 times smaller compared to the oxygen drop of 23.95±4.85% calculated for a stocked raceway. The oxygen drop calculated for the unstocked raceway is within the standard deviation of the oxygen drop shown for a stocked raceway. Therefore oxygen consumption by biofouling or mineralization within the raceways was neglected in the calculation of oxygen consumption by fish (Eq. 6.8).

6.4.3.- Oxygen consumption by fish

The aim of the present study was to estimate the oxygen consumption of juvenile California halibut under farm-like conditions. Comparing the data obtained in this study with those for other flatfish is difficult. Most measurements on flatfish species have been done under laboratory conditions, generally in small respirometers under starved conditions or following controlled rations (Table 6.4). Brown (1980) and Brown et al. (1984) reported oxygen consumption by turbot measured at 15 °C in farm-like conditions, but that study was limited to a short observation (30 minutes) done one hour after the final feed of the day (at 16:00 h). In that study fish were fed hourly from 11:00 h to 16:00 h, and feed was offered during 10 minutes on each occasion. Under those conditions, oxygen consumption rates for turbot were from 11.71 to 3.6 g O_2 / kg fish d (Table 6.4) which was very similar to the rates found here for juvenile California halibut (Table 6.3). Juvenile California halibut between 3.2 and 165.6 g mean mass studied in the present research had a mean daily oxygen consumption rate between 3.9 and 12.4 g O_2 / kg fish d when reared at 21.6 ± 1.0 °C (Table 6.3). Mallekh and Lagardere (2002) reported 5.64 g O_2 / kg fish d as the maximum oxygen consumption rate when fed turbot were forced to swim at temperatures of 18 to 22 ° C. For California halibut, a maximum rate of 4.6 g O_2 / kg fish d (Table 6.3) can be expected at 21.6 ± 1.0 °C, however the fish were not forced to swim as described above for turbot.

Table 6.4.- Oxygen consumption rate (M_{day}, g O_2/kg fish d) for flatfish species under laboratory and culture conditions.

Species	Temp °C	Feed	W g	Fish N	Obs. Period h	M_{day} g O_2/kg fish d	Author
Hirame	20	no	438	1	4 – 8	1.00	Honda, 1988
				2	4 - 8	0.90	
				3	4 - 8	0.83	
				5	4 - 8	0.89	
Turbot[1]	7 - 8	Yes	177	farm tank	0.5	1.23	Brown et al., 1984
	9 - 10	Yes	165	farm tank	0.5	2.06	
	11 - 12	Yes	143	farm tank	0.5	2.20	
	13 - 14	Yes	123	farm tank	0.5	3.43	
	15 - 16	Yes	4	farm tank	0.5	11.71	
	15 - 16	Yes	44	farm tank	0.5	5.79	
	15 - 16	Yes	229	farm tank	0.5	3.60	
Turbot	18.7	No	107 - 185	6	16	1.37	Waller, 1992
	22.4	No	107 - 185	6	21	1.39	
Flounder	15	No	395	4	n.a.	5.15	Duthie, 1982
Common dab	15	No	395.8	4	n.a.	4.11	
Lemon sole	15	No	221.3	4	n.a.	3.95	
Atlantic halibut[2]	10	Yes	1023	1	16	5.75	Davenport et al., 1990
Lemon sole[2]	10	Yes	594	1	16	3.70	

n.a. = no available

1. Pellet ration is not indicated. The diet was moist pellet with a protein level of 64%.

2. Fish fed to satiation on skinned and filleted saithe flesh.

6.4.4.- Oxygen consumption relative to the feed offered

For a number of species (Freeman et al., 1967; Davenport et al., 1990; Malloy and Targett, 1991; Fonds et al., 1995; Mallekh et al., 1998; Talbot et al., 1999), the rate of feeding declines as fish mass increases, but increases with temperature. California halibut feeding rates in the present study were set according to the feeding protocol at the Hatchery and Nursery facilities (section 6.2.7). The feeding protocol reduces feeding ration from 3.0 to 0.7 % BW with increase in fish size.

In the present study, California halibut juveniles consumed between 0.31 and 1.4 g O_2/g feed. Feed used here was manufactured for salmonids. For salmonid feeds, the oxygen demand is assumed to be about 0.2 g O_2 / g feed (Meade, 1991). Timmons et al. (2001) assumed that fish metabolism requires 0.25 g O_2/g feed. The values obtained for California halibut are larger than the ones reported and used for design purposes. The high values of oxygen demand relative to the amount of may be linked to the low feed rations used, as the largest oxygen consumption rates per unit of feed occurred when the smallest feed rations were offered (ie. 9.2 g fish fed at 2 %BW consumed less oxygen than 11.8 g fish fed at 1.2 %BW; 117.9 g fish fed at 1.2 %BW consumed less oxygen than 115.7 g fish fed at 0.7 %BW; 131.6 g fish fed at 1.2 %BW consumed less oxygen than 132.4 and 133.0 g fish fed at 0.7 %BW Table 6.3).

Jobling and Davies (1980) showed that the rate of oxygen consumption was strongly correlated with meal size and duration. Alsop and Wood (1997) explained that the ingestion of feed is followed by an increase in metabolic rate in most animals, a phenomenon known as specific dynamic action (SDA). In teleost fish, SDA is thought to represent all the metabolic expenditures associated with the nutritive process including the energy required for ingestion, digestion, absorption, metabolic transformation of nutrients and growth (Jobling, 1981b; Brown and Cameron, 1991; Lyndon *et al.* 1992; Jobling, 1994). Alsop and Wood (1997) said that the sites where oxygen is utilized to support swimming metabolism (skeletal muscle, predominantly red muscle) are different from the sites where oxygen is utilized to support the SDA effect (liver, intestine). Alsop and Wood (1997) suggest that there is a feeding threshold at which the amount of oxygen available to the muscles for swimming performance is relocated for SDA metabolic processes There are studies supporting Alsop and Wood (1997) findings, in which satiated fish were found to reduce swimming activity and turning into a resting posture (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991). Feeding results in a decrease in red muscular blood flow, while increasing visceral blood flow in resting fish (Axelsson *et al.* 1989; Axelsson and Fritsche, 1991).

Therefore it is possible that California halibut (within the same mass range and independent of stocking density) fed at a higher feeding ration in this research reduced its swimming metabolism due to SDA effect, and therefore the

spontaneous activity was minimized while resting on the bottom of the tank. On the other hand, fish fed at a lower ratio had also a smaller SDA effect, which might have had not inhibited their swimming and spontaneous activity, allowing for extra energy expended in the search for feed.

Consequently, it is likely that California halibut fed at a lower ratio were more active, and the oxygen consumed due to fish activity was erroneously attributed to the feed eaten resulting in larger oxygen consumption by feed. Also from Table (6.4) oxygen consumption by starved fish is as high as that of fed fish. According to the literature, SDA is the only factor that can be directly attributed to the level of digestible energy in the diet (Brown, 1980; Alsop and Wood, 1997). In addition, Kristiansen et al. (2003) reported that when Atlantic halibut were reared in high density culture tanks, the fish resting on the bottom were continuously being disturbed by moving and landing fish, and were thus deprived of resting and stimulated to move or start swimming. Brown (1980) reported that the oxygen consumed by turbot above the resting level was not only due to SDA, but also fish activity caused a considerable increase in oxygen consumption. Furthermore, the costs of social factors (aggression, overcoming turbulence created by neighbouring fish) may also influence oxygen consumption (Christiansen and Jobling, 1990). Therefore further research is needed to determine with exactitude the oxygen consumption of California halibut due to feed.

6.4.5.- Apparent effect of fish stocking density on oxygen consumption

This is the first work where an apparent effect of stocking density on routine oxygen consumption rates in California halibut with a size ranging between 3.2 and 14.2 g under farm-like conditions has been observed. On the other hand, no apparent effect on oxygen consumption rates was observed for fish between 110.5 and 165.6 g stocked between 94 and 296% PCA (Table 6.3).

Results from this project show that there were differences in mean M_{day} and $M_{F\text{-}day}$ rates for California halibut ranging between 11.8 and 14.2 g stocked between 107 and 316% PCA (Table 6.3). In addition, the 12.5, 12.8, and 11.8 g juvenile California halibut were also followed for several weeks in a study performed to determine the effect of stocking density on their growth (see Chapter V for more details). After six weeks the 12.5, 12.8, and 11.8 g fish had a percentage of relative mass gain (PWG) of 83%, 75%, and 65% for final stocking densities of 152%, 281%, and 395% PCA, respectively. Therefore, increasing stocking density in this particular case resulted in decreases in oxygen consumption rate and, in the long term, a decrease in fish growth.

Although water flow for all raceways was adjusted to have a similar dissolved oxygen concentration in the effluent (~ 5 mg/L), it is possible that at the high stocking densities used, the benthic behavior of the fish and the shallowness of the water caused some of the fish to be exposed to lower oxygen concentrations (hypoxia). Consequently, it is possible that to save energy, California halibut

subjected to hypoxia were less active than those under normoxia. Voyer and Morrison (1971) reported oxygen consumption rates for 13-23 g winter flounder of 1.68 g O_2 / kg fish d at 3.2 mg O_2/L and 2.4 O_2 / kg fish d at 6.3 mg O_2/L, both at temperatures ranging from 10 to 20 °C. A similar reduction in respiration rates was also reported for English sole, *Parophrys vetulus*, reared in reduced dissolved oxygen environments (Boese, 1988). In addition, a reduction in growth under low dissolved oxygen conditions has been described in a number of fish including flatfish (Pederson, 1987; Peterson and Pihl, 1991; Tallqvist et al., 1999; Pichavant et al., 2000). Significantly lower growth rates in juvenile winter flounder were reported for a dissolved oxygen environment cycling from 2.5 to 6.4 mg/L at 18.7 °C compared to fish held at about 6.0 mg/L (Bejda et al., 1992). Turbot (120 g average mass; 80 fish/tank; 10 to 15 L/min water flow) fed to satiation and exposed for 45 days to 7.2 mg O_2/L (95% saturation), 5.0 mg O_2/L (65% saturation), and 3.5 mg O_2/L (45% saturation) showed a reduction of feed intake with a decrease in environmental dissolved oxygen (Pichavant et al., 2000). The reduction of feed intake at 3.5 and 5.0 mg O_2/L resulted in a growth reduction of 25% in 45 days, compared to fish held at 7.2 mg O_2/L (Pichavant et al., 2000).

6.4.6.- Diel pattern of oxygen consumption.

For aquaculture purposes, it is sometimes recommended that average oxygen consumption rates be used for calculating oxygen requirements for a fish farm (Colt and Orwicz, 1991; Bergheim et al., 1993). However, fish metabolism is partly regulated by endogenous diel rhythms and exogenous diurnal cycles

(Haijin et al., 1997). Furthermore, diel variations in metabolic consumption of oxygen in fish are dominated by feed and feeding frequency (Brett and Zala, 1975). For production conditions, the ratio of peak/average oxygen consumption rates ranges from 1.2 to 1.4 (Colt and Orwicz, 1991). Increasing the number of feedings per day and lengthening the feeding period can significantly reduce diel oxygen consumption fluctuation (Colt and Orwicz, 1991). In the present study California halibut were offered a continuous feeding during 12 h (within the light phase) resulting in maximum daily oxygen consumption rates that were between 1.2 and 1.5 times the average daily values (Table 6.3). Oxygen consumption of the larger fish tested (110.5 to 165.6 g fish), showed a less marked diel variation.

The typical diel cycle shown by California halibut had peak oxygen consumption during the feeding period in day light and relatively low and falling oxygen consumption after feeding was stopped until the following morning (Fig. 6.4 to Fig. 6.25). These results were different from those reported for starved juvenile Japanese flounder (5.4 to 8.3 g) which significantly increased oxygen uptake during the dark hours (Haijin et al., 1997). Patterns of increased oxygen consumption rates during light periods and linked to feeding times have been reported for sockeye salmon (Brett and Zala, 1975), Atlantic salmon (Bergheim et al., 1991; Bergheim et al., 1993), white sturgeon (Thomas and Piedrahita, 1997), and sea bass (Tudor, 1999).

The diel variation in dissolved oxygen consumption by California halibut caused an occasional reduction below the targeted 5 mg O_2/L at the effluent zone. Depending of the length and frequency of the periods of hypoxia, a reduction in growth and an increase in mortality might be expected (Bejda et al., 1992; Tallqvist et al., 1999; Taylor and Miller, 2001). Therefore effective water distribution must be kept or designed within culture vessels holding high stocking densities to avoid exposing the fish to lower oxygen concentration. Diel dissolved oxygen patterns with periods of reduced concentration may cause severe stress in fish, leading to appetite reduction and growth depression (Withworth, 1968; Carlson et al., 1980; Bejda et al., 1992). Winter flounder kept under diurnally fluctuating dissolved oxygen between 2.5 and 6.5 mg/L (average of 5.1 mg/L) showed significant differences in growth when compared to fish held under a constant oxygen concentration of 6.5 L/min, but grew faster than fish held at a constant 2.5 mg/L (Bejda et al., 1992). It has been shown that when mean long term dissolved oxygen concentrations were kept above critical levels for post smolt Atlantic salmon, large fluctuations in dissolved oxygen did not affect growth when compared to stable dissolved oxygen concentrations (Forsberg and Bergheim, 1996). In this research with California halibut, it was not possible to determine the effect of diurnal oxygen concentrations changes on fish growth rates, which will be critical to the design and management of future commercial facilities. When designing an aquaculture facility for California halibut the maximum oxygen consumption recorded from the diel cycles has to be considered; otherwise a design based on average daily rates of oxygen

consumption might lead to periods of hypoxia during periods of maximum oxygen consumption and spatial variations in the raceways.

6.4.7.- Oxygen consumption rate and fish size relationship

Small fish generally consume more oxygen per unit of mass than large individuals (Fivelstad et al., 1999). However, in intensive aquacultural systems, the oxygen consumption rate might be influenced by numerous parameters other than fish mass. Therefore, the oxygen consumption equations shown in Fig. 6.26 can only be assumed to be valid for the conditions tested in this research, since the model (Eq. 6.15) does not account for the activity or feeding levels.

The data presented in Table 6.5 and plotted in Fig. 6.27 show a relationship between oxygen consumption and mean fish mass for juvenile California halibut and other species. The oxygen consumption rate of juvenile California halibut decreased with increasing mean mass (Fig. 6.26, Fig. 6.27). Similar findings were reported by Duthie (1982) for three flatfish species; flounder, common dab, and lemon sole (Table 6.5). Similar results were also found for turbot reared under farm conditions (Brown et al., 1984) and at laboratory scale (Waller, 1992; Mallekh and Lagardere, 2002) (Table 6.5). The mass exponents estimated for California halibut in the present study range from -0.20 to -0.25. The mass exponent for California halibut were similar to those estimated for turbot (-0.18 to -0.28) under farm culture conditions (Brown, 1980) and less negative than for starved hirame (-0.64) under laboratory conditions (Haijin et al., 1997) (Table 6.5).

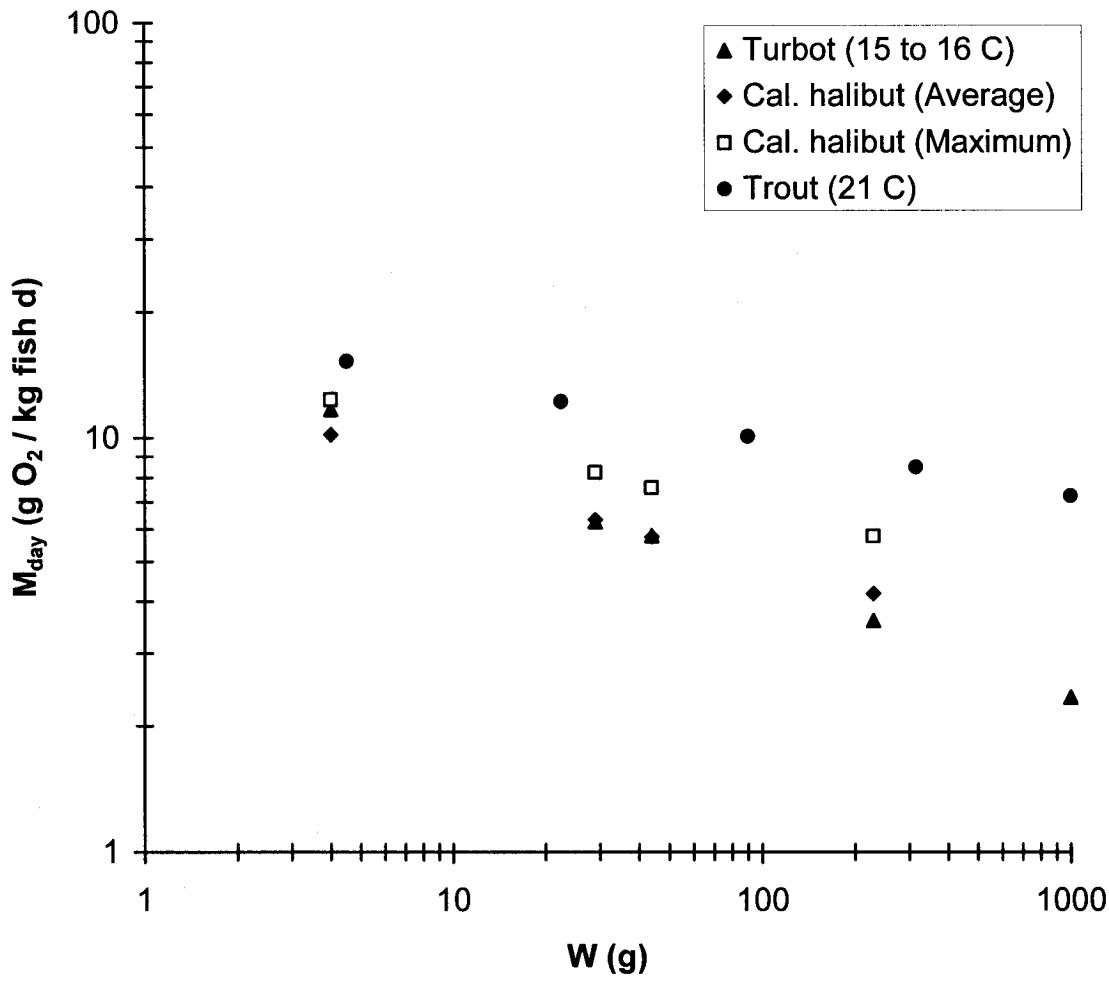

Figure 6.27.- Relationship between oxygen consumption rates and mean body mass for trout (Liao, 1971), turbot (Brown 1980), and California halibut (this study).

Table 6.5.- Coefficients for the function $M_M = a\ W^b$ that describes the relationship between oxygen consumption rate (g O_2/kg fish d) and fish mass (g).

Species	Temp °C	Feed	A	b	r^2	Mass range g	Author
California halibut	21.56	Yes	15.077	-0.2452	0.83	3.2 to 165.6	This study for average
	21.56	Yes	17.266	-0.2033	0.79	3.2 to 165.6	This study for maximum
Turbot	7 – 8	Yes	3.3475	-0.1837	0.9776	4 to 686	Brown, 1980
	9 – 10	Yes	6.1289	-0.2509	0.9025	5 to 1000	
	11 – 12	Yes	8.4717	-0.2567	0.9145	4 to 1000	
	13 – 14	Yes	11.344	-0.2742	0.928	4 to 1000	
	15 – 16	Yes	17.175	-0.2891	0.9988	4 to 1000	
Turbot	n.a.	No	6.8821	-0.2997	0.9411	1.5 to 1073	Waller, 1992
Hirame	20	No	0.413	-0.643	0.966	2.4 to 67.5	Haijin et al., 1997
Flounder[1]	15	No	0.115	0.812	0.990	8 to 1000	Duthie, 1982
Common dab[1]	15	No	0.138	0.634	0.973	2 to 500	
Lemon sole[1]	15	No	0.168	0.783	0.981	2 to 500	
Trout[2]	21	n.a.	18.754	-0.138	n.a	n.a.	Liao, 1971
Atlantic salmon	15.5 to 16.5	yes	16.5354	-0.187	0.37	1.29 to 11.84	Fivelstad et al, 1999

n.a. no available

1. M_M is expressed as mg O_2/h

2. Species of trout tested were rainbow, lake, Kamloop, splake, cutthroat, and steelhead.

6.5.- CONCLUSIONS

The most relevant conclusions from the present chapter are:

a) Overall, California halibut juveniles showed a decrease in oxygen consumption with an increase in body mass, independent of stocking density and feed offered. It appears that oxygen consumption relative to feed applied was affected by the feeding ration and it was independent from the fish stocking density. At a lower feeding ration a larger oxygen consumption relative to feed applied was estimated, but there is no clear explanation of this observation.

b) In spite of the above overall conclusion, the oxygen consumption for California halibut weighing between 11.8 and 12.5 g was inversely proportional to the stocking density. However, in the long term the fish stocked at the highest densities, which had a smaller oxygen consumption, resulted in lower biomass gain. This phenomenon might be due to fish located right on the bottom of the tank being exposed to lower oxygen concentrations, which affected their oxygen consumption rate and growth. Hence, special care has to be addressed when designing or studying the hydraulics of the culture tank. Oxygen consumption for California halibut weighing between 110.5 and 165.6 g was not affected by stocking density.

c) The typical diel rhythm shown by California halibut had a peak consumption rate during the feeding period in daylight and a relatively low and falling consumption rate after feeding was stopped until the following

morning. The diel rhythm resulted in maximum daily oxygen consumption rates that were between 20 and 50 % greater than average daily values. The maximum oxygen consumption rates for juvenile California halibut can be expressed by $M_{day} = 17.266\ W^{-0.2033}$. This equation is valid for California halibut ranging between 3.2 and 165.6 g. This equation does not consider other environmental and biological factors that might also affect the oxygen consumption of fish under intensive culture.

d) Finally, the determinations of oxygen consumption in this research for California halibut in farm-like conditions provide valuable information of the oxygen requirement of these fish in an aquacultural setting. This information can be used for designing and sizing of a rearing facility for the intensive culture of California halibut.

6.6.- FURTHER RESEARCH

Further studies are recommended in the following areas:

- Determine feed ration and energetic composition for maximum fish growth related to oxygen consumption.

- Determine if California halibut larger than the ones used in this research can be stocked at higher densities than smaller fish without affecting growth rates and survival. This proposal is based on the knowledge that small fish have a higher mass-specific oxygen consumption than large ones.

- Determine how oxygen concentration affects oxygen consumption by California halibut.

- Determine for how long and with what frequency California halibut can be exposed to low oxygen concentrations without affecting growth rates and survival.

- Determine how hydraulics (shape, depth, flow patterns) and stocking density affect the distribution of dissolved oxygen in culture tanks.

CHAPTER VII

DETERMINATION OF AMMONIA AND UREA EXCRETION RATES OF CALIFORNIA HALIBUT UNDER CULTURE

7.1.- INTRODUCTION

Most flatfish are grown in land-based facilities and are stocked at high densities per unit of water volume or of bottom area. The high stocking densities used generally require high water flow rates into the tanks to supply the oxygen requirements and to carry out the metabolic byproducts (Lawson, 1995). If oxygen requirements can be satisfied by aeration or oxygenation devices, the next major water quality concern are the metabolic byproducts excreted by the fish, such as ammonia and urea (Colt and Armstrong, 1981; Handy and Poxton, 1993; Tanaka and Kadowaki, 1995). Metabolic waste concentration may reach high levels in recirculating water, thereby increasing the possibility of lethal or sublethal effects from nitrogenous byproducts on fish (Brunty et al., 1997; Person-Le Ruyet at al., 1997).

In the design and optimization of water recirculation and treatment technologies, it is important to know the nitrogen excretion rates. Although studies have been performed with some flatfish species (Birkett, 1969; Jobling, 1981; Kikuchi et al., 1991; Dosdat et al., 1996; Carter et al., 1998; Varter and Bransden, 2001), there has been no report on the nitrogenous excretion of California halibut. The

purpose of this study is to determine the nitrogenous excretion as TAN and urea-N for California halibut under farm-like conditions within a recirculating water system. More generally, data on nitrogen metabolism in California halibut are of great importance in the determination of the potential environmental impact of future aquaculture operations.

7.1.1.- Literature review

Almost all teleost fishes excrete ammonia as the predominant nitrogenous waste, but they also excrete a small amount of urea, usually around 20% of the total nitrogen excreted (Handy and Poxton, 1993; Dosdat et al., 1996; Chadwick and Wright, 1999). Both ammonia and urea excretion amount to more than 95% of the total nitrogen excreted, and the rest consists of about 1% of non-protein nitrogen (Trimethylamine-N, Trimethlamine-N, Creatine-N, and Creatinine-N) and about 3% of undetermined nitrogen (Wood, 1993; Dosdat et al., 1996).

7.1.1.1.- Ammonia

Ammonia in water exists as two compounds: ionized (NH^+_4) and un-ionized (NH_3) ammonia (Timmons et al., 2001). The acid dissociation for ammonia may be expressed as:

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad \text{(Eq. 7.1)}$$

Researchers have agreed that total ammonia nitrogen should be used to express the sum of the nitrogen present in the two compounds (NH_4^+ and NH_3). Therefore, total ammonia nitrogen (TAN) includes both the un-ionized ammonia nitrogen ($NH_3 - N$) and the ionized ammonium nitrogen (NH_4^+ - N).

The relative concentrations of the two ammonia forms depend on water pH, temperature, and salinity (Masser et al., 1999; Timmons et al., 2001). The mole fraction of un-ionized ammonia nitrogen (a) can be estimated from the expression (Fivelstad et al., 1995):

$$a = \frac{1}{1+10^{((pK_a+S_K+0.0324(24.85-T))-pH)}} \qquad \text{(Eq. 7.2)}$$

where pK_a = negative log of the acid dissociation constant for total ammonia at 25 °C

S_K = correction term for the salinity

T = temperature (°C)

Emerson et al. (1975) presented the following expression to calculate the acid dissociation constant for total ammonia:

$$pK_a = 0.09018 + \frac{2729.92}{T+273.15} \qquad \text{(Eq. 7.3)}$$

And the correction term for salinity is given by (Whitfield, 1974):

$$S_K = \frac{2.3116 * S}{1000 - 1.005109 * S} \qquad \text{(Eq. 7.4)}$$

where S = salinity (g/L).

The concentration of un-ionized ammonia nitrogen can be calculated as:

$$NH_3 - N = a * TAN \qquad \text{(Eq. 7.5)}$$

where NH_3-N = concentration of un-ionized ammonia nitrogen (mg/L)

TAN = concentration of total ammonia nitrogen (mg/L)

Un-ionized ammonia has been reported to be much more toxic to fish than ionized ammonia (Alderson, 1979; Colt and Armstrong, 1981; Meade, 1985: Soderberg and Meade, 1991; Handy and Poxton, 1993; Randall and Tsui, 2002). According to Eq. 7.1 the mole fraction of un-ionized ammonia can be diminished by decreasing pH (Randall and Tsui, 2002), by decreasing temperature (Timmons et al., 2001), and by increasing the salinity (Alabaster et al., 1979), therefore decreasing the toxicity for a given TAN. Randall and Tsui (2002) stated that "*TAN toxicity varies with pH but the effects of temperature and salinity are much less important.*"

7.1.1.2.- Ammonia toxicity in fish

Toxic effects of TAN on fish physiology, which depends on the percentage of the un-ionized form present in solution, may include a decrease of growth rate, diminished fertility and weakened immunity, as well as increased vulnerability to changes in temperature and oxygen levels (Handy and Poxton, 1993). Chronic exposure to TAN damages fish gills, reducing the epithelial surface area available for gas exchange (Larmoyeaux and Piper, 1973; Smart, 1976; Soderberg et al., 1984). Furthermore, gill damage caused by ammonia exposure may contribute to reduced growth by reducing oxygen consumption (Handy and Poxton, 1993).

Lethal concentrations at 96-h LC_{50} for juvenile sea bass, seabream, and turbot range from 1.7 to 2.7 mg NH_3 – N/L (Wajsbrot et al., 1991; Person-Le Ruyet et al., 1995). The long term effects of TAN in marine fish are not very well documented; however, it has been reported that concentrations between 0.09 and 3.35 mg NH_3 – N/L cause an acute toxic effect for seawater species (Handy and Poxton, 1993), while safe levels for growth are between 0.05 and 0.2 mg NH_3 – N/L (Person-Le Ruyet et al., 1997).

For 3 g turbot fry, it has been reported that wet mass decreased linearly with increasing concentrations of un-ionized ammonia when they passed the threshold concentration of 0.11 mg NH_3 – N/L (Alderson, 1979). Juvenile turbot (~20 g) reduced their food intake when un-ionized ammonia was over 0.117 mg

NH_3 – N/L, and a reduction in body mass gain per day occurred when the level was over 0.108 mg NH_3 – N/L (pH 8, 16 °C, 28 g/L salinity) (Rasmussen and Korsgaard, 1996). Hence the threshold level seems to be around 0.11 mg NH_3 – N/L for optimal growth of turbot fry and juveniles. In sole and seabream juveniles, the thresholds for no growth were between 0.38 – 0.77 (pH range 6.9 – 7.9) and 0.5 mg NH_3 – N/L, respectively (Alderson, 1979; Wajsbrot et al., 1993). Person-Le Ruyet et al. (1997) reported that for 13, 23, and 104 g turbot, reared in water having in the effluent over 80% oxygen saturation (~6 mg/L), the growth was not affected at concentrations of 0.21, 0.18, 0.09 mg NH_3 – N/L, respectively, while growth stopped immediately for all groups above 0.8 mg NH_3 – N/L (~pH 8, ~17 °C, 34.5 g/L salinity). Turbot survival was not affected at levels below 0.4 mg NH_3 – N/L (13 mg TAN/L) (Person-Le Ruyet et al., 1997).

7.1.1.3.- Total ammonia production rate

Total ammonia nitrogen excretion rates are directly related to dietary nitrogen and protein intake in fish (Haskell, 1955; Liao and Mayo, 1974; Rychly, 1980; Beamish and Thomas, 1984; Handy and Poxton, 1993; Wagner et al., 1995). Haskell (1955) found that the average total ammonia production rate in trout hatcheries was 32 g/kg feed fed per day. The relationship between offered nitrogen and TAN excretion has been reported for some flatfish species to be between 12 and 25 g/kg feed fed (Table 7.1) (Jobling, 1981; Kikuchi et al., 1991; Dosdat et al., 1995). Immediately after feeding, the rate of TAN excretion of

Table 7.1.- Excretion rates of nitrogenous compounds from marine fish.

Species	Type	W	Feed	Ration % of the	P	Nitrogen Excreted	Excretion rate	Excretion rate[1]	T	Notes	Source
		g	%BW	ad libitum	%	(%)	mg/kg h	g/kg feed	°C		
Turbot	TAN	13.5		0		E	2.05			Fish fed twice daily	Dosdat et
				20	54	9.77	2.91			(10:00 and 16:00)	al., 1995
				36	54	11.35	3.72				
				52	54	11.11	4.22				
				68	54	11.19	4.78				
				84	54	12.5	5.66				
				100	54	14	6.61				
	Urea-N	13.5		0		E	0.4			Fish fed twice daily	
				20	54	3.7	0.72			(10:00 and 16:00)	
				36	54	4.31	1.03				
				52	54	3.42	1.06				
				68	54	3.09	1.15				
				84	54	3.45	1.39				
				100	54	3.32	1.49				
Hirame	TAN	189 to	0.5		47	31	5.38	25.80	20	Fed once/d	Kikuchi et
		575	1		47	24.8	7.50	18.00		Large feed ration	al., 1991
			1.5		47	16.5	7.92	12.67		Increased the time for TAN and	
	Urea-N	189 to	0.5		47	4.45	0.75	3.60	20	Urea-N excretion	
		575	1		47	3.18	0.96	2.30			
			1.5		47	2.18	1.04	1.67			

Table 7.1.- (continuation)

Species	Type	W	Feed	P	Nitrogen excreted	Excretion rate	Excretion rate[1]	T	Notes	Source
		g	%BW	%	(%)	mg/kg h	g/kg feed	°C		
Hirame	TAN	2.4 to 9.5	0		E	5		16	Fish fed once a day (8:30 to 9:00)	Kikuchi et al., 1995
			1.9 to 3.0	54	22.8	19.2	18.91	16		
			0		E	7.5		20		
			1.6 to 2.9	54	24.3	20	19.70	20		
			0		E	9.16		25		
			1.2 to 3.0	54	26.1	21.9	21.57	25		
	Urea-N	2.4 to 9.5	0		E	1.04		16		
			1.9 to 3.0	54	2.4	1.96	1.93	16		
			0		E	1.08		20		
			1.6 to 2.9	54	2.7	2.21	2.18	20		
			0		E	1.09		25		
			1.2 to 3.0	54	3.6	2.83	2.79	25		
Hirame	TAN	1.6 to 6.5	3	47	23.2	19.9	15.92	20	Fish fed once a day (9:00)	Kikuchi, 1995
		31 to 56	1.5	47	21	8.6	13.76	20		
		264 to 517	0.5	47	31.9	5.4	25.92	20		
	Urea-N	1.6 to 6.5	3	47	2.9	2.5	2.00	20		
		31 to 56	1.5	47	3.8	1.58	2.53	20		
		264 to 517	0.5	47	4.5	0.75	3.60	20		

Table 7.1.- (continuation)

Species	Type	W	Feed	P	Nitrogen excreted	Excretion rate	Excretion rate[1]	T	Notes	Source
		g	%BW	%	(%)	mg/kg h	g/kg feed	°C		
Turbot										
	TAN	13.9	1.48	55.2	21	10.15	16.46	20	Fed twice/d	Dosdat et al., 1996
	TAN	179	0.43	55.2	20.5	3.08	17.19	16	Fed once/d	
	Urea-N	13.9	1.48	55.2	5.1	2.51	4.07	20	Fed twice a day	
	Urea-N	179	0.43	55.2	5.94	0.89	4.97	16	Fed once a day	
Greenback Flounder	TAN	1.62	1	49	87	7.9	0.50	17	10 fish, fed morning	Verbeeten et al., 1999
		1.8	1		83.3	9.12	0.71		10 fish, fed morning	
		3.82	2		50.9	10	7.00		10 fish, fed morning	
		4.48	2		65.21	12	11.56		10 fish, fed morning	
		4.43	3		36.9	11.2	15.83		10 fish, fed morning	
		5.75	3		42	11.6	27.61		10 fish, fed morning	
	Urea-N	1.62	1	49	21.7	1.96	0.12	17	10 fish, fed morning	
		1.8	1		11.58	1.3	0.10		10 fish, fed morning	
		3.82	2		49.1	9.7	6.79		10 fish, fed morning	
		4.48	2		34.8	6.4	6.17		10 fish, fed morning	
		4.43	3		63	19.1	26.99		10 fish, fed morning	
		5.75	3		57.9	16	38.09		10 fish, fed morning	

Table 7.1.- (continuation)

Species	Type	W	Feed	P	Nitrogen excreted	Excretion rate	Excretion rate[1]	T	Notes	Source
		g	%BW	%	(%)	mg/kg h	g/kg feed	°C		
Atlantic Salmon	TAN	2050	0.9	44.5	66.8	6.00	16.10	12	Feeding 24 h at 3 feed per h	Fivelstad et al., 1990
		3200 - 3500	0.6	44.5	66	2.76	12.00	10	Feeding 24 h at 2 feed per h	
		3800 - 4200	0.4	42.5	64.2	3.24	20.00	4	Feeding 8:00 to 20:00 at 1 feed per h	
		59	1.6	45	Na	3.42	5.30	11	Feeding 24 h at 4-5 feed per h	
	Urea-N	2050	0.9	44.5	10.1	0.90	2.50	12	Feeding 24 h at 3 feed per h	
		3200 - 3500	0.6	44.5	10.4	0.42	1.80	10	Feeding 24 h at 2 feed per h	
		3800 - 4200	0.4	42.5	8.3	0.42	3.00	4	Feeding 8:00 to 20:00 at 1 feed per h	
		59	1.6	45	Na	na	Na	11	Feeding 24 h at 4-5 feed per h	

W = fish biomass

P = diet protein content

T = temperature

E = endogenous excretion

na = no available

[1] Nitrogen excretion rates to feed eaten (g/kg feed) were estimated from author's data

hirame fed 0.5, 1.0, and 1.5%BW with a diet containing 47% protein was 3 to 4 times that of starved fish (Kikuchi et al., 1991). Rates of TAN excretion in turbot, at any hour, were reported to be higher at higher feeding rates (Dosdat et al., 1995). In addition, Jobling (1981) studying plaice and Kikuchi et al. (1995) hirame indicated that the rate of nitrogen excretion was dependent upon temperature and the amount of digestible nitrogen administered. There are reports that fish can excrete between 60 and 95% of feed nitrogen and that 1 kg of feed can produce between 19.5 to 300 g of TAN (Rychly, 1980; Meade, 1985; Mires and Amit, 1990; Masser et al., 1992; Wright, 1993; Begum et al., 1994; Wagner et al., 1995). Furthermore, it has been reported that fish body mass did not affect the nitrogen excretion rates per unit mass for hirame between 15 and 575 g (Kikuchi et al., 1990).

The production rates of total ammonia have been reported to exhibit a diurnal pattern (Brett and Zala, 1975; Forsberg, 1996). Several factors such as fish species, fish size, feed quality, nitrogen intake, ration size, and ration distribution may affect the daily pattern, the amplitude and the time of appearance of peaks of TAN excretion rates after feeding (Lied and Braaten, 1984; Ramnarine et al., 1987). Effects of the ration level on the daily pattern of TAN excretion were studied by Jobling (1981) with plaice and by Kikuchi et al. (1991) with hirame. They reported that the duration of elevated rates of TAN excretion was extended with an increase in ration level. Kikuchi et al. (1991) indicated that the TAN excretion rates of hirame fed at 0.5 and 1.0%BW rations dropped to the level of

starved fish after 12-24 and 30-36 h, respectively; the fish fed at 1.5%BW had a TAN excretion that was still higher than that of starved fish after 36-48 h.

Excretion rates may vary according to unimodal or polymodal rhythms, depending on the frequency of feeding (Kaushik, 1980; Kaushik and Cowey, 1991). According to Poxton and Lloyd (1989), the distribution of the daily feed in two meals per day led to the lowest overall diel amplitude excretion of TAN by the European eel (*Anguilla anguilla*) and the lowest peak concentration occurred when these feedings were widely spaced. In turbot fed one meal per day, at every feeding level tested, it was found that postprandial TAN excretion rate increases rapidly to a single peak before returning slowly to the initial level (Dosdat et al., 1995). When turbot were fed two meals a day, two peaks were noticeable, as reported in rainbow trout (Kaushik, 1980) and in cod (Ramnarine et al., 1987). The feeding period and feeding strategy were seen to influence the daily rhythm of metabolite production in Atlantic salmon, which showed a lack of a typical daily cycle when the fish were under a regime of 24 h frequent feeding and light (Fivelstad et al., 1990).

With respect to the maximum rate of excretion it has been shown that the maximum rate had no apparent increase at higher ration levels (Jobling, 1981; Kikuchi et al., 1991). Ramnarine et al. (1987) suggested that there is a maximum physiological capability for the biochemical processes related with protein

assimilation and nitrogen excretion, hence the relationship between ration level and nitrogen excretion should be curvilinear.

7.1.1.4.- Urea

Urea is the only other nitrogen compound excreted by fish in significant quantities, besides ammonia. Dosdat et al. (1996) considered it to be an advantage for aquaculture operations that a large proportion of the nitrogen excretion in turbot was in the form of urea. Their conclusion was based on the fact that urea is much less toxic than TAN, and therefore that, within a recirculating system stocked at high density, a healthier rearing environment must be expected. However, although urea is reported as non-toxic to the fish, it can be rapidly hydrolyzed to ammonia and carbon dioxide in culture systems if urea-hydrolizing bacteria are present (Colt and Armstrong, 1981; Pedersen et al., 1993). There have been more than 200 urea-hydrolizing species of bacteria described, including both Gram-positive and Gram-negative (Pedersen et al., 1993). Therefore the ammonia produced from the urea hydrolysis should be considered a part of the overall ammonia budget in the culture system (Kikuchi, 1995). Consequently, urea plus TAN excretion rates have to be considered when designing the biofiltration component within a recirculating system.

Most fish species cannot tolerate high environmental ammonia levels but some species are ammonia-tolerant and have a variety of strategies to avoid ammonia toxicity. Some fish have the capacity to shift their nitrogen excretion from

ammonia to the less toxic urea if they are exposed to environments with high ammonia concentrations (Olson and Fromm, 1971; Randal et al., 1989; Walsh et al., 1990; Tanaka and Kadowaki, 1995; Saha et al., 2001; Kajimura et al., 2002; Randall and Tsui, 2002).

Marine sediments have been reported to have a high content of ammonia (Lomstein et al., 1989), which might make it difficult for benthic fish, such as flatfish, to excrete ammonia. There is a single report describing a significant increase both in plasma urea-N levels and in daily urea-N excretion rates occurring when juvenile turbot were exposed to high ambient ammonia concentrations (Person-Le Ruyet et al., 1997; Person-Le Ruyet et al., 1998).

7.1.1.5.- Urea production rate

In addition to ammonia, fish can excrete metabolic nitrogen as urea (Randall and Wright, 1987). In some fish species, urea can make a substantial contribution to nitrogen excretion (Table 7.1) (Davenport et al., 1990; Kikuchi et al., 1990; Dosdat et al., 1996; Verbeeten et al., 1999). Urea concentration is often expressed as urea-N (Kikuchi, 1995; Verbeeten et al., 1999).

A post-prandial increase in TAN excretion by fish was systematically observed (Brett and Zala, 1975; Kaushik, 1980; Ramnarine et al., 1987), whereas no such pattern has been noted in urea excretion (Brett and Zala, 1975; Fivelstad et al., 1990). Although in early studies urea-N excretion was not found to correlate with

nitrogen intake in the same way as ammonia-N excretion (Brett and Zala, 1975; Fivelstad et al., 1990; Verbeeten et al., 1999), several authors have now demonstrated a linear relationship in flatfish species (Kikuchi et al., 1991; Dosdat et al., 1995; Carter et al., 1998; Verbeeten et al., 1999). Urea usually represents 10 to 23% of the total ammonia and urea excretion for hirame and turbot (Kikuchi et al., 1992; Dosdat et al., 1996) and up to 60% for greenback flounder (Verbeeten et al., 1999) living in good quality waters (Table 7.1).

Urea-N daily excretion of small (< 5 g) greenback flounder were affected by ration and also by feeding regime, particularly time of feeding (Verbeeten et al., 1999). Peaks of urea excretion occurred during the light and dark phase for greenback flounder fed in the morning and in the evening, respectively (Verbeeten et al., 1999). In addition, turbot, whether fed or not, showed a single high excretion peak which presented a time lag inversely related to the feeding level (or nitrogen intake) (Dosdat et al., 1995). In turbot, urea-N plasma concentrations rose quickly after feeding to a very high level (77 mg/L), before decreasing 11-13 h after feeding (Dosdat et al., 1996). Diurnal changes in the excretion rate of urea-N in hirame were observed to follow the same pattern as TAN excretion: increasing after the first feeding (10:00 h) and reaching a maximum rate at about 2-4 h after the last meal (18:00 h) at 16 °C, and 1-2 h after the last feeding (18:00 h) at 19 °C (Tanaka and Kadowaki, 1995).

7.2.- EXPERIMENTAL DESIGN

Experiments on nitrogen excretion of California halibut were carried out under farm-like conditions. In this section a description of the system used for the excretion studies is presented along with the culture techniques practiced for the intensive culture of this fish. A detailed description is also given for the procedures followed to determine the excretion rates of TAN and urea-N of California halibut.

7.2.1.- Fish stock

Experiments were performed between November 25, 2002 and February 6, 2003. Two generational groups, 2001 and 2002, of juvenile California halibut bred in captivity were used in this experiment. Both generations were reared intensively in a recirculating system at the University of California Davis (Appendix A.1). Experiments with the 2001 fish were carried out in a recirculating system at the Bodega Marine Laboratory, and with the 2002 fish at the recirculating system at the University of California Davis. In this experiment, rates of TAN and urea-N excretion were measured for California halibut of 4.2, 7.2, 10.4, and 20.1 g, which belonged to Generation 2002 and for fish of 112 and 199 g mean mass which belonged to Generation 2001. Water temperature was 21.5 ± 0.3 ^{0}C and salinity 30 ± 1.2 g/L for the UC Davis facility, and 19.4 ± 1.0 ^{0}C and 32.5 ± 1.0 g/L at the Bodega Bay facility. Water temperature and salinity were within the optimum ranges recommended for California halibut culture (Gadomski

and Caddell, 1991; Madon, 2002). Light was provided by overhead fluorescent tubes on a 16 L: 8 D (L=light; D=dark) photoperiod (Boeuf and Le Bail, 1999; Klokseth and Oiestad, 1999).

7.2.2.- Feeding and feeds

California halibut feeding rates used in the present study were set according to the feeding record protocol at the Hatchery (at Davis) and Nursery facilities (at Bodega Bay) (Table 7.2). All the fish stocks were weighed every other week and feeding rations were adjusted at that time. Feed amount was set at each weighing and kept until the following weighing. Feed was distributed by automatic 12 h belt feeders (09:00 to 21:00) during the daylight hours (07:00 to 23:00) 7 d/wk.

Diets used were manufactured by Silver Cup® and EWOS® Canada Limited for salmonids (Table 7.3). According to the manufacturer's information, the pellets have protein and fat levels which deliver maximum growth potential for all Pacific salmonids (EWOS) and for trout (Silver Cup). Silver Cup® fry pellets 1 and 2 mm were used to feed fish less than 7 g. The EWOS® pellets 2 and 3 mm were used to feed fish larger than 10 g.

Table 7.2.- Feed ration for California halibut as a percentage of fish biomass (%BW). Feed ration was pre-determined by the Recirculating California Halibut Hatchery Management Protocol.

W (g)	Feed ration %BW
3 - 6	3.0 – 2.5
6 – 25	2.0 – 1.0
25 - 75	1.5 – 1.0
75 – 200	1.2 - 0.5

Table 7.3.- Nutritional characteristics of feed used to growth California halibut as supplied by the manufacturers.

Contents	SILVER CUP	EWOS
Crude protein	45 %	43 %
Crude fat	19 %	14 %
Crude fiber	3 %	2 %
Ash	12 %	9 %
Moisture	Less than 10 %	8 %
Vitamin A	10000 IU/Kg	3000 IU/Kg
Vitamin D3	500 IU/Kg	3000 IU/Kg
Vitamin E	250 IU/Kg	150 IU/Kg

7.2.3.- Experimental tank

The culture tanks used for the excretion experiment were under an open-flow mode operation at the recirculating systems located at Davis and Bodega Bay. However, given the low rates of ammonia and urea production relative to the flow rate through the tanks, it was not possible to measure accurately changes in concentration from the influent to the effluent. Hence, the tanks were periodically operated in a batch mode (Levenspield, 1999; Mihelcic, 1999) to allow the measurement of ammonia and urea production rates.

Two types of tanks were used in this research: raceway (at Davis) and circular (at Bodega Bay). The ammonia and urea concentration level in the effluent from both of these tanks are expected to represent the nitrogen excretion rate for the entire fish biomass held within the tank (Colt and Armstrong, 1981). This analysis assumes that the TAN and urea-N excretion does not depend on the ambient ammonia level within the range of concentrations present in the tanks.

7.2.3.1.- Test system at Davis

The hatchery at the University of California Davis has four raceways, 27.5 cm in width, 241 cm in length and ~5 cm of water depth. A 19 cm long quiescent zone was located at the effluent section. Cylindrical nets (5 mm mesh aperture) were placed within the raceways at the influent and effluent pipes. During the excretion experiment the raceways were isolated from the recirculating system and were

operated in a batch mode (Fig. 7.1). The water level during an experimental run was controlled by an internal standpipe (Fig. 7.1). A batch system was established by the turning off of the influent water coming from the head tank (see Appendix A.1 for details), and by the opening of the valve from the effluent pipe (Fig. 7.1). The effluent water poured through a 50 µm filter bag and into a reservoir (Fig. 7.1). Within the reservoir, strong aeration was provided and the water was pumped back at 15 L/min to the head of the raceway (Fig. 7.1).

7.2.3.2.- Test system at Bodega Bay

At the nursery facility at the Bodega Bay Marine Laboratory, there were six circular tanks (see Appendix A.1 for details). Circular tanks were 99 cm in internal diameter and 50.8 cm in depth. Two of the circular tanks were isolated from the recirculating system and turned into a batch operation mode during the experimental excretion studies (Fig. 7.2). The water level during the experiment was controlled by an external standpipe (Fig. 7.2). A batch system was operated by turning off the influent water coming from the head tank (see Appendix A.1 for details), and by closing the valve from the effluent pipe (Fig. 7.2). A screen structure was installed in the effluent section of the circular tank (Fig. 7.2). The effluent water poured through a 50 µm filter bag and into a reservoir (Fig. 7.2). Within the reservoir strong aeration was provided and the water was pumped back at 15 L/min to the surface of the circular tank (Fig. 7.2).

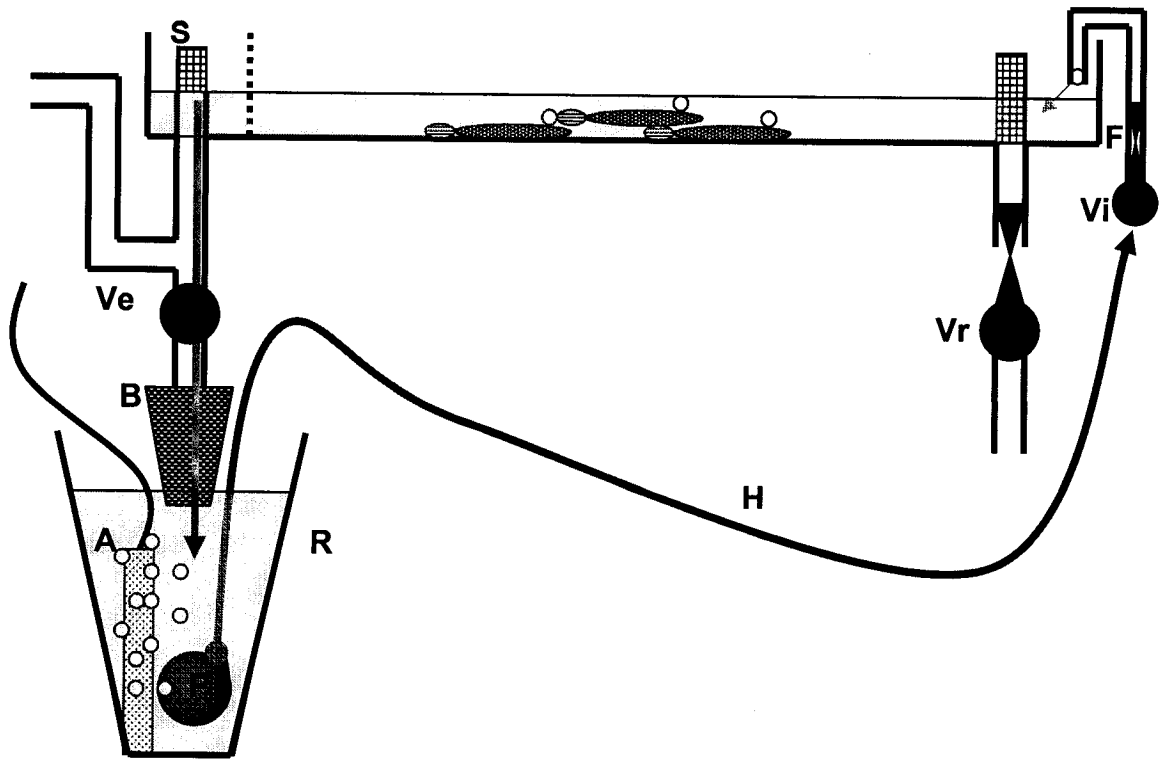

Figure 7.1.- Schematic diagram of a raceway used for the N excretion studies and its operation. Water in the raceway flows from right to left. The normal flow to the raceway is closed at valve Vr. The recirculated influent flow during a test is regulated with a valve (Vi) and a flowmeter (F). With full opening of the effluent valve (Ve), the flow from the raceway is directed towards a 50 µm bag filter (B) and poured into a reservoir (R). Within the reservoir the water is aerated (A) and pumped (P) back through a hose (H) to the head of the raceway. Both influent and effluent are covered with a screen. Water level within the raceway is controlled during the experiment phase with an internal standpipe (S). The schematic diagram is not to scale. Water samples were taken from the water pouring into B from Ve.

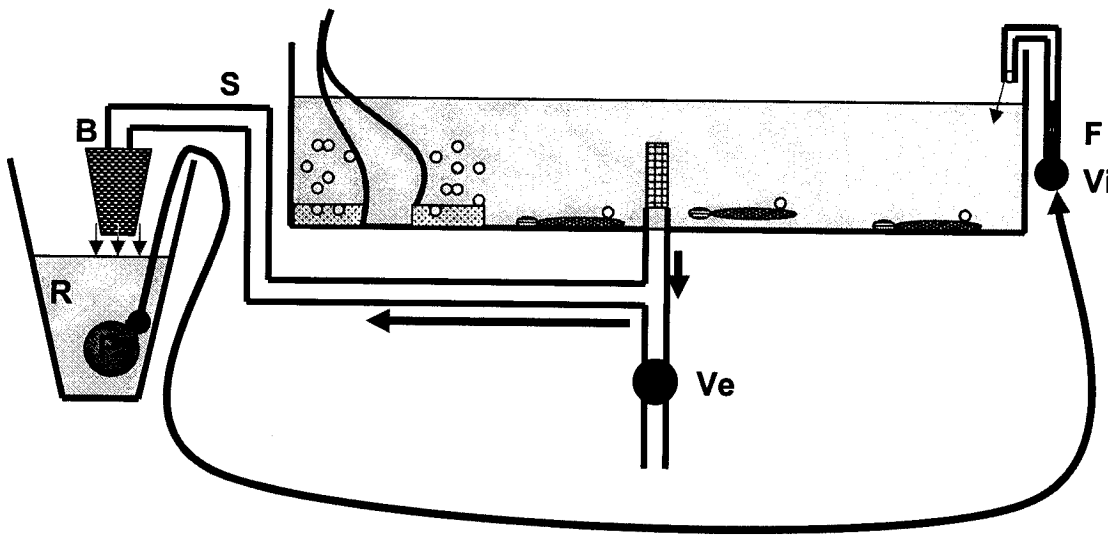

Figure 7.2.- Schematic diagram of a circular tank used for the N excretion studies and its operation. The influent flow is regulated with a valve (Vi) and a flowmeter (F). By fully closing of the effluent valve (Ve), the outlet flow from the circular tank is directed towards a 50 µm bag filter (B) and poured into a reservoir (R). The water level within the circular tank is controlled by an external standpipe (S). From the reservoir, the water is pumped (P) back to the circular tank. The effluent pipe within the tank is covered with a screen. The schematic diagram is not to scale.

7.2.4.- Experimental protocol

Fish tanks were operated as usual during the experiment, so the TAN and urea-N excretion data were collected under farm-like conditions. Tanks were cleaned every morning at 9:00 h, by gentle scrapping of the surface walls under the water level. Feeding was resumed about 9:30 h and for the next 12 hours through loading of the belt feeders with the corresponding amount of feed. General fish culture conditions are shown in Table 7.4.

Sampling of raceways and circular tanks for TAN and urea-N were performed over three 8 h sampling periods (09:00 – 17:00; 17:00 – 01:00; 01:00 – 09:00). To determine rates of TAN and urea-N excretion, the fish culture tanks were set from flow-through to batch operation mode. During batch mode, the tanks were sampled at time 0 and every 2 h for TAN and urea-N. Upon completion of an 8 h period, the tanks were switched back to flow-through operation mode for 8 to 16 h. Experiments were conducted over 5 d so that each tank was sampled over each of the three 8 h periods, with 8 or 16 h between samplings. Each time frame was analyzed twice for each raceway. No mortalities were registered during and up to one week of finalizing the experiment. Water temperature was 21.5 ± 0.3 ^{0}C and salinity 30.0 ± 1.2 g/L for the UC Davis facility, and 19.4 ± 1.0 ^{0}C and 32.5 ± 1.0 g/L at the Bodega Bay facility. Effluent dissolved oxygen was always above 5 mg/L.

Table 7.4.- Fish culture conditions. TAN and urea-N excretion rate tests were performed on six average fish masses. Tests were done under farm-like conditions. Stocking densities ranged from 171 to 466 percent of coverage area.

	Experimental date					
	November 25 to 30, 2002		January 8 to 11, 2003		February 3 to 6, 2003	
Facility	U C Davis		U C Davis		Bodega Bay Marine Laboratory	
N	889	518	973	442	80	45
TW (g)	3708	3722	10113	8896	8933	8933
W (g)	4	7	10	20	112	199
PCA%	245	198	466	316	205	171
%BW	2.1	2.4	1.2	1.2	1.05	1.05
Type of feed	SC 1	SC 2	EWOS 2	EWOS 2	EWOS 5	EWOS 5
% Crude fat	19	19	14	14	14	14
% N in feed	45	45	43	43	43	43

7.2.5.- Water quality measurements

Triplicate 10 mL water samples were collected for TAN and urea-N at time 0, and again every 2 h in each sampling period from the water pouring into the bag filter. The water removed from the vessels for sampling was replaced immediately after sampling with water from the recirculating system. The total volume for a raceway operating under batch mode was about 71 L, and for a circular tank was about 290 L.

TAN concentrations were measured colorimetrically with Hach™ reagents by the salycilate method (Hach method #8155), which is specific for seawater samples (Verbeeten et al., 1999). Urea-N concentrations were measured by the diacetyl monoxime method (Koroleff, 1983; Price and Harrison, 1987; Mulvenna and Savidge, 1992) adapted from a method employed in clinical medicine (http://w3.whosea.ord/micro/4.htm) (Appendix A.3). Water samples for TAN were analyzed immediately according to Hach™ methodology. Water samples for urea-N were stored at 4 ^{0}C in assay test tubes and analyzed immediately after the respective 8 h time frame period ended. Both the TAN and urea-N measurements are colorimetric methods, and a Hach™ Odissey spectrophotometer (model DR/2500) was used to determine the respective concentrations.

7.2.6.- Fish sampling

Fish were counted and weighed as a group to obtain their total biomass (B) and estimate their mean mass immediately after an experimental sequence was finished.

Mean fish biomass (W, g per fish) was estimated as:

$$W = B / N \qquad \text{(Eq. 7.6)}$$

where B = total fish biomass (g)

N = number of fish

Culture density as percentage of coverage area (PCA, %):

$$PCA = 100 * (TSA * N) / A \qquad \text{(Eq. 7.7)}$$

where TSA = average fish total surface area (cm^2)

A = tank surface area used for culture (cm^2).

PCA was estimated for the 2002 fish generation (<30 g) using a relationship between TSA and W (Appendix A.2):

$$TSA_{2002} = 7.0801 * W^{0.6056} \qquad \text{(Eq. 7.8)}$$

Similarly, a PCA for 2001 fish (> 66 g) was also estimated using a relationship between TSA and W (Appendix A.2):

$$TSA_{2001} = 11.224 * W^{0.5716} \qquad \text{(Eq. 7.9)}$$

7.2.7.- Estimation of dissolved TAN and urea-N production rates in a closed system

Following the methodology described by several authors (Enging and Carter, 2001), TAN and urea-N excretion rates were determined by mass balance analysis under farm-like conditions. The vessels were considered as batch systems open to the atmosphere (Fig. 7.3).

The mass balance of dissolved TAN and urea-N (Fig. 7.3) for a constant volume batch system can be described with (Mihelcic, 1999):

$$\frac{dC}{dt} = r_{diff} + r_x \qquad \text{(Eq. 7.10)}$$

where, $\frac{dC}{dt}$ = change in concentration of dissolved TAN or urea-N in the tank over a period of time (mg / L h)

r_{diff} = rate of diffusion of TAN or urea-N between water and the atmosphere (mg / L h)

r_x = net rate of production of TAN or urea-N (mg / L h)

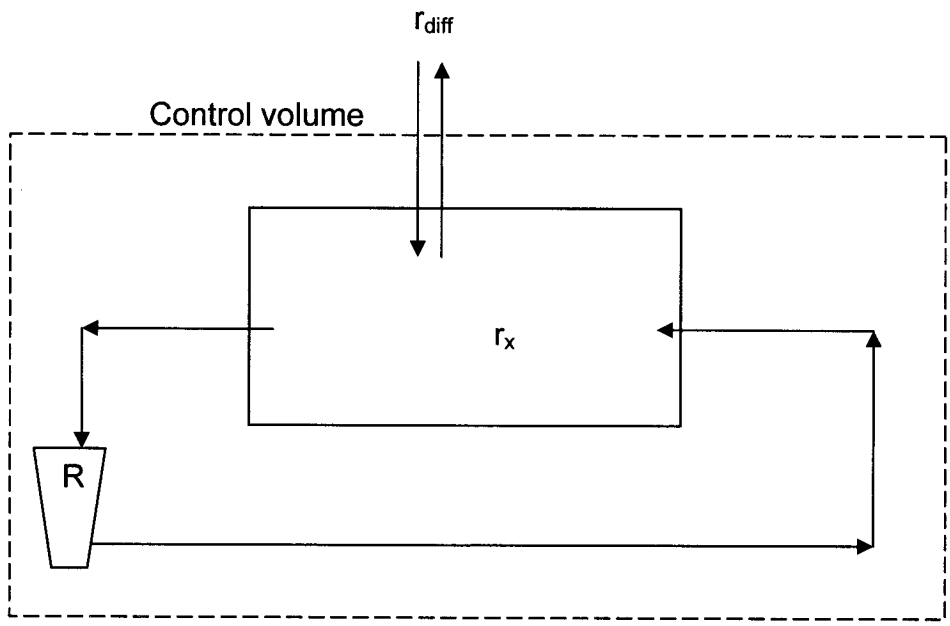

Figure 7.3.- Schematic diagram for raceway (Fig. 7.1) and circular (Fig. 7.2) tanks. Terms are as defined in the text. Water samples for TAN and Urea-N were taken from the water pouring into the reservoir (R).

The rate of diffusion of TAN between the water and the atmosphere was assumed to be negligible, based on observations by Thomas and Piedrahita (1998). Therefore Eq. 7.10 can be rearranged to solve for the production rate:

$$r_x = \frac{\Delta C}{\Delta t} = \frac{C_{t+1} - C_t}{t_1 - t} \qquad \text{(Eq. 7.11)}$$

where, C_{t+1} = TAN or urea-N concentration at time t +1

C_t = TAN or urea-N concentration at time t

The production rate (r_x) can be positive or negative, both for TAN and urea-N. A negative production rate means that the compound of interest is disappearing from the solution. Urea production rate can be expected to be negative if it is hydrolyzed to TAN in culture systems by the action of urea-hydrolizing bacteria (Colt and Armstrong, 1981; Pedersen et al., 1993). TAN can be expected to be negative if it is nitrified to nitrite-N (Timmons et al., 2001). A TAN concentration in the culture media higher than the TAN concentration in fish blood can reduce or even inhibit TAN excretion, and therefore a negative production rate can be also expected (Olson and Fromm, 1971; Walsh et al., 1990; Tanaka and Kadowaki, 1995; Kajimura et al., 2002; Randall and Tsui, 2002).

7.2.8.- TAN or Urea-N production rates calculation

TAN and urea-N production rates were calculated using (TAN) or urea-N as the reference material in the mass balance equations. TAN or urea-N were sampled

from the batch system every 2 h within a frame time of 8 h. TAN or Urea-N concentrations were expressed in units of mg/L.

The hourly mass specific rate for TAN or urea-N excretion (M_M as mg TAN or Urea-N/ kg fish per h) can be expressed as:

$$M_M = \frac{r_x * V * 1000}{B} \qquad \text{(Eq. 7.12)}$$

where, V = batch system volume (L)

1000 = factor to convert g fish to kg

The maximum hourly mass specific rate for TAN or urea-N excretion (M_{Mmax} as mg TAN or urea-N/kg fish per h) can be expressed as:

$$M_{Mmax} = \frac{r_{xmax} * V * 1000}{B} \qquad \text{(Eq. 7.13)}$$

where, r_{xmax} = maximum net rate of production of TAN or urea-N (mg/L h)

1000 = factor to convert g fish to kg

There were three experimental time frames of 8 h, to complete an observation time equivalent to 24 h. In each experimental time frame four M_M values were calculated every 2 h. Hence twelve M_M values were obtained per day. Each M_M value was multiplied by 2 and added to obtain an overall daily excretion value (M_{Mday}, mg TAN or urea-N/kg fish per d):

$$M_{Mday} = 2 * \sum_{1}^{12} M_M \qquad \text{(Eq. 7.14)}$$

The feeding ration per day (F, g feed per day) is defined as:

$$F = \frac{\%B}{100} B \qquad \text{(Eq. 7.15)}$$

where, %B = percentage of fish biomass per day (1/d)

And the nitrogen ration per day, (F_N, g N-feed per day) (Forsberg, 1996):

$$F_N = F * P * 0.16 \qquad \text{(Eq. 7.16)}$$

where, P = the decimal fraction of protein in the diet

0.16 = nitrogen content in protein

Therefore, the daily feed specific rate for TAN or urea-N excretion (M_{Fday} as g TAN or Urea-N/ kg feed) can be expressed as:

$$M_{Fday} = \frac{M_{Mday} * B}{F} \qquad \text{(Eq. 7.17)}$$

And the daily nitrogen specific rate for TAN or urea-N excretion (M_{Fday} as g TAN or Urea-N/ kg N-feed) can be expressed as:

$$M_{Fday} = \frac{M_{Mday} * B}{F_N} \qquad \text{(Eq. 7.18)}$$

The maximum rate for TAN and urea-N excretion per fish ($M_{FISH-MAX}$, mg TAN or urea-N/fish per h), can be expressed as:

$$M_{FISH-MAX} = \frac{M_{MMAX}}{W} c \qquad \text{(Eq. 7.19)}$$

where, c = factor to convert unit of mass (1000 kg/g)

The daily rate for TAN and urea-N excretion per fish ($M_{FISH-DAY}$, mg TAN or urea-N/fish per d), can be expressed as:

$$M_{FISH-DAY} = \frac{M_{Mday}}{W} c \qquad \text{(Eq. 7.20)}$$

7.2.9.- Relationship of TAN and urea-N excretion rate to fish mass

The relationship between fish mass and metabolism usually can be expressed by an exponential equation (Paloheimo and Dickie, 1966). It has been confirmed that the relationship for nitrogen excretion can be shown in the same form for plaice (Jobling, 1981) and hirame (Tanaka and Kadowaki, 1995). Therefore the metabolic data from this research were pooled using:

$$M_M = a * W^b \qquad \text{(Eq. 7.21)}$$

where M_M = specific rate of TAN or urea-N excretion by mass or by fish unit (mg TAN or urea-N / kg fish per h or per d; mg TAN or urea-N / fish per h or per d)

a and b are species specific constants

7.2.10.- Data analysis

Daily excretion rates presented in this study accounted for all the hourly rates. Maximum and hourly excretion rates by biomass or by fish were calculated from

the respective hourly net rate (r_x). A t-test was used to compare the daily, maximum, and hourly TAN or urea-N excretion rates. Statistical comparisons were conducted using SAS statistical software (release 8.02 Level 02M0). Significances were analyzed using Duncan's multiple range test and Tukey's studentized range test at a significance of 0.05 (Dean and Voss, 1999).

A linear regression analysis was used to study the relationship between dietary nitrogen intake and TAN or urea-N excretion rates (Dean and Voss, 1999).

7.3.- RESULTS

The hourly and daily TAN and urea-N excretion rates were obtained for a 24 h cycle. The 24 h cycle was subdivided in three 8 h time frame: 9:00 – 17:00 h, 17:00 – 1:00 h, and 1:00 – 9:00 h. The maximum concentrations of TAN and urea-N within each 8 h frame time will be presented first. Thereafter, the diel TAN and urea-N excretion patterns will be presented by plotting the hourly excretion rates for each of the three 8 h time frames. Finally, daily TAN and urea-N excretion rates will be presented.

7.3.1.- TAN and urea-N concentration

TAN and urea-N concentrations within each 8 h time frame time are presented in Table 7.5. The highest concentrations of TAN for 4.2 and 7.2 g fish were measured between 9:00-17:00 h and 17:00-1:00 h, while the highest urea-N

Table 7.5.- Maximum TAN concentration within the tanks at each oh the three frame times. The highest TAN concentration within each of the 8 h frame test for each replicate was chosen, and the values were averaged. The number of replicates was two.

W (g)	4.2	7.2	10.4	20.1	111.7	198.5
Maximum TAN (mg/L)						
9:00 to 17:00 h	2.42±0.16	2.30±0.01	4.55±1.01	3.47±0.66	0.36±0.03	0.37±0.08
17:00 to 1:00 h	2.23±0.14	2.53±0.75	6.90±0.42	6.20±0.66	1.73±0.01	1.75±0.01
1:00 to 9:00 h	1.14±0.41	0.84±0.21	5.08±1.54	3.99±1.87	1.13±0.01	1.15±0.19
Maximum Urea-N (mg/L)						
9:00 to 17:00 h	0.21±0.09	0.21±0.07	0.92±0.21	0.78±0.20	0.12±0.03	0.15±0.02
17:00 to 1:00 h	0.58±0.02	0.55±0.13	1.00±0.01	1.03±0.06	0.37±0.01	0.35±0.01
1:00 to 9:00 h	0.47±0.10	0.60±0.21	1.03±0.22	0.86±0.13	0.21±0.01	0.16±0.03

concentration was between 17:00-1:00 h and 1:00-9:00 h. For 10.4 and 20.1 g fish the highest TAN concentrations were observed between 17:00–1:00 h, while the urea-N concentration was similar for the three 8 h period. Finally, for the 111.7 and 198.5 g fish the highest TAN concentrations occurred between 17:00-1:00 h and 1:00-9:00 h, while the highest urea-N concentrations occurred between 17:00-1:00 h (Table 7.5). Overall, there is no clear pattern as to the time when peak TAN and urea-N concentrations took place.

7.3.2.- Diel pattern of hourly TAN and urea-N excretion rates

Daily TAN and urea-N excretion patterns are presented in Figures 7.4 through 7.9. Juvenile California halibut were fed continuously from ~9:00 to 21:00 h during the light phase period. Hourly TAN excretion rate for 4 g (Fig. 7.4) and 7 g (Fig. 7.5) fish started to increased 4 to 6 h after the daily feeding was started; for 10 g (Fig. 7.6) and 20 g (Fig. 7.7) TAN excretion rate increased 2 to 4 h after feeding; and for 112 g (Fig. 7.8) and 199 g (Fig. 7.9) fish TAN excretion rate increased 8 to 10 h after feeding started. Hourly TAN excretion rate for all fish sizes reached a maximum around 19:00-21:00 h. Hourly urea-N excretion rate for all fish was greater ($p<0.05$) than TAN excretion between 1:00-3:00 h or between 3:00-5:00 h. Hourly urea-N excretion was not significantly different ($p>0.05$) from TAN excretion rate several times in the day for all fish sizes, in particular at times 9:00-11:00 h and 17:00-19:00 h.

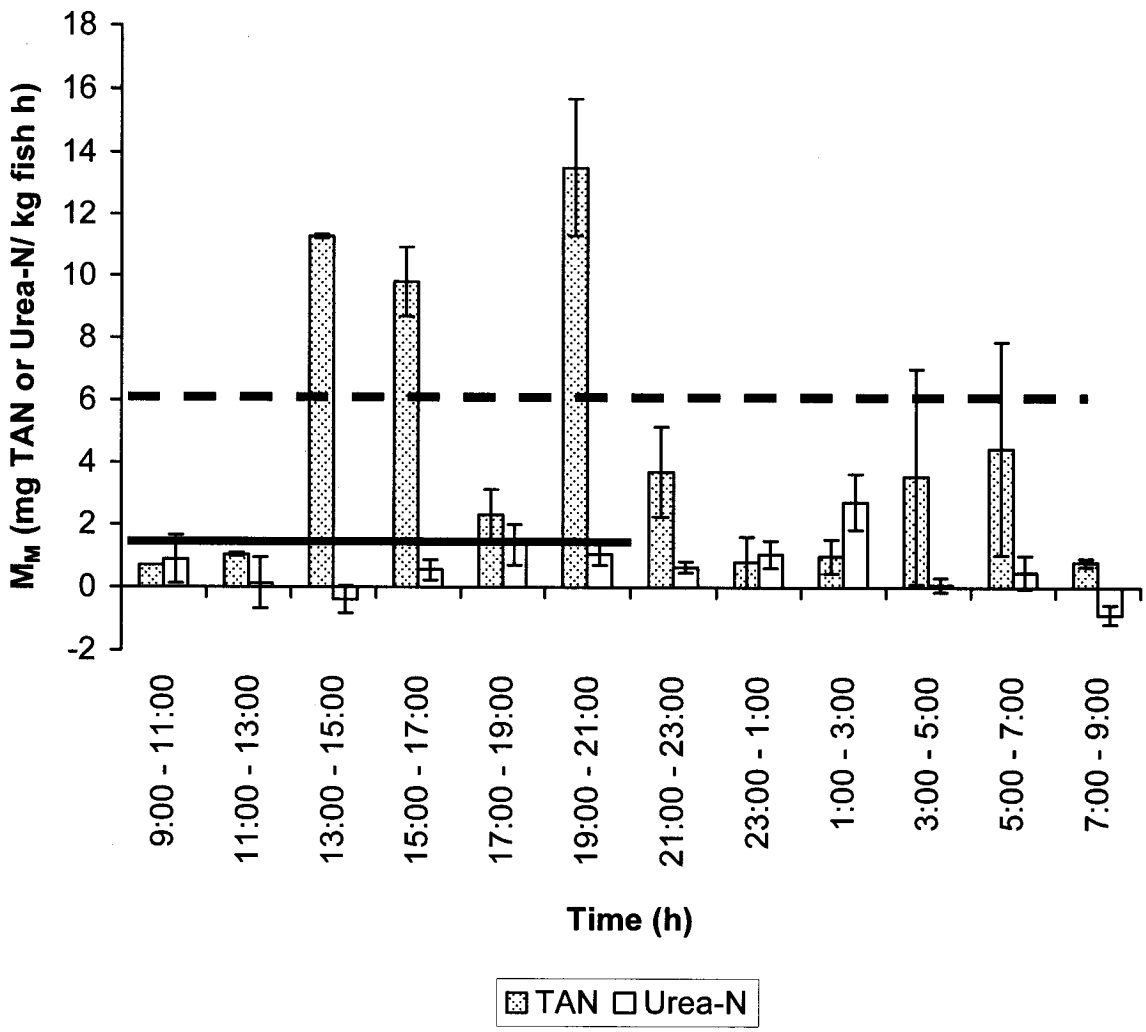

Figure 7.4.- Hourly TAN and urea-N excretion profile for 4 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

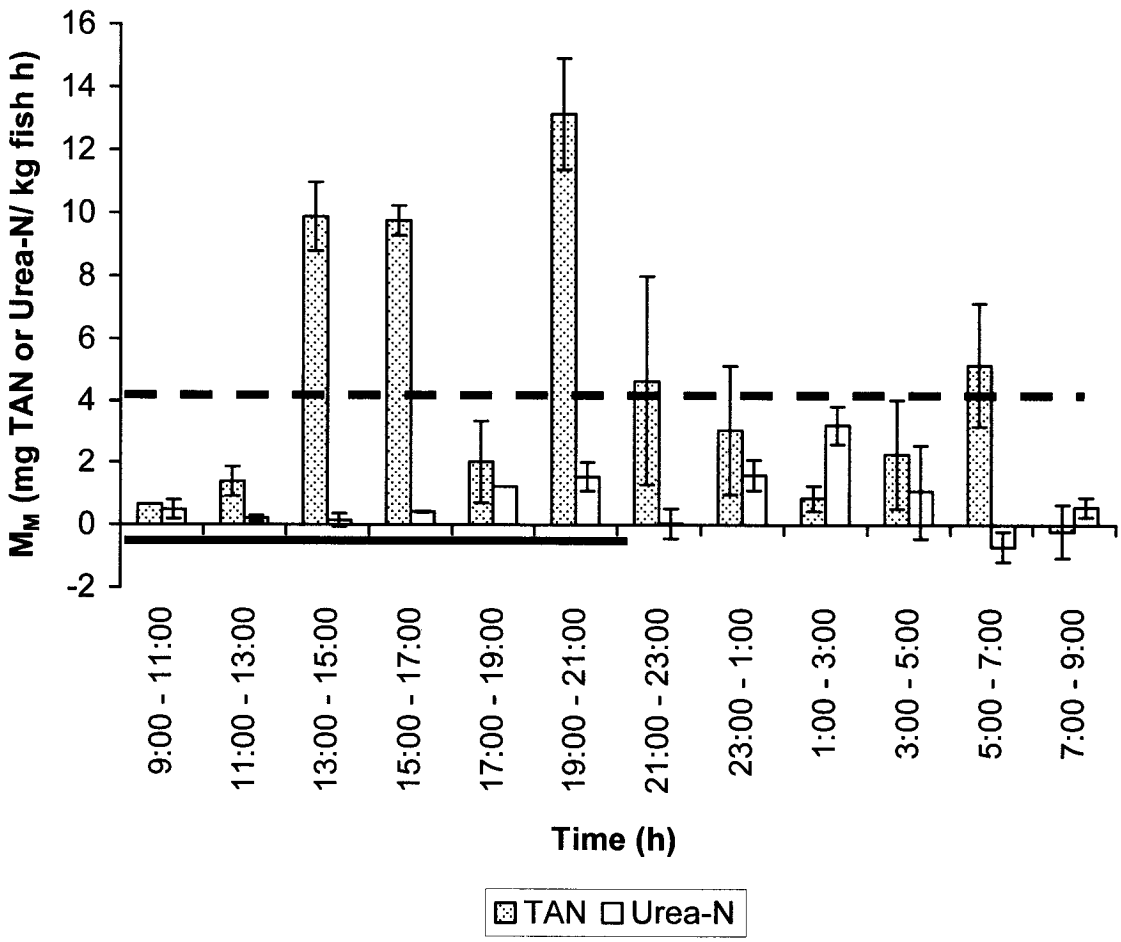

Figure 7.5.- Hourly TAN and urea-N excretion profile for 7 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

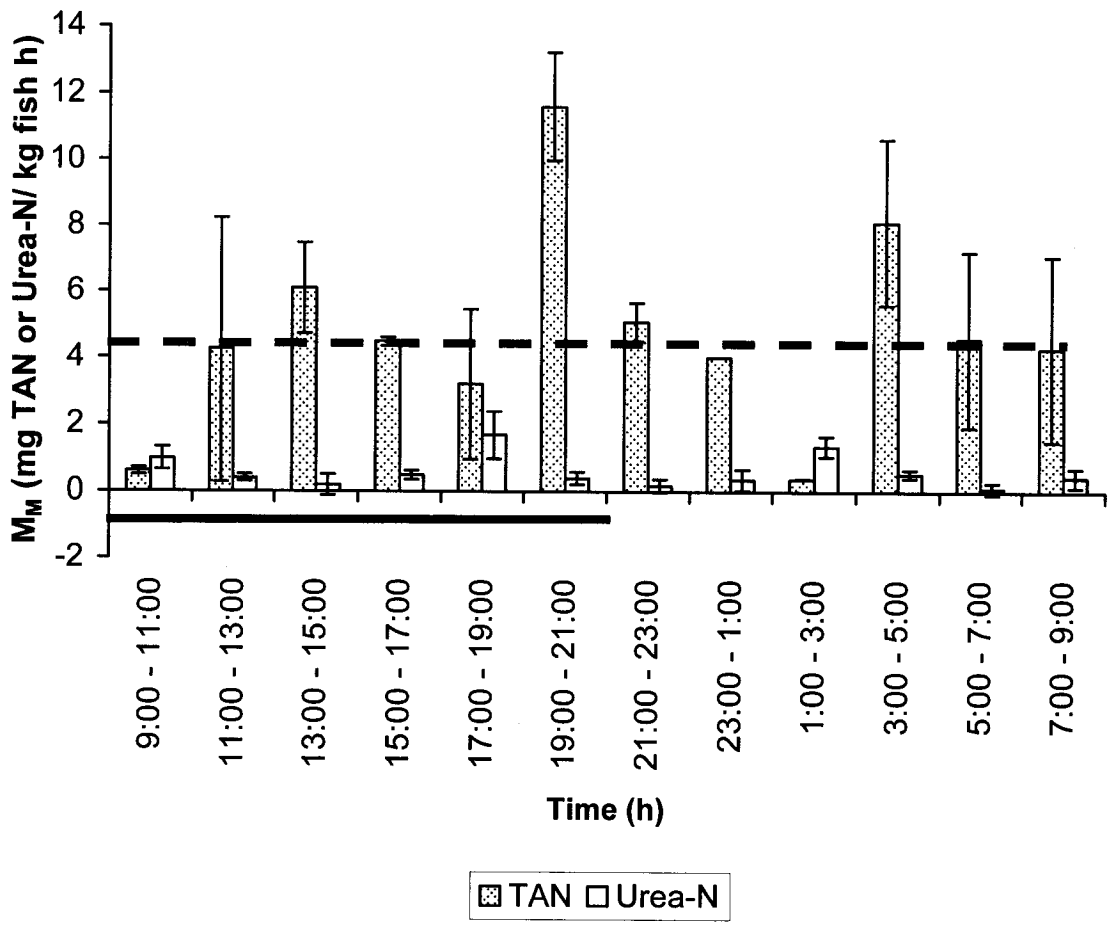

Figure 7.6.- Hourly TAN and urea-N excretion profile for 10 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

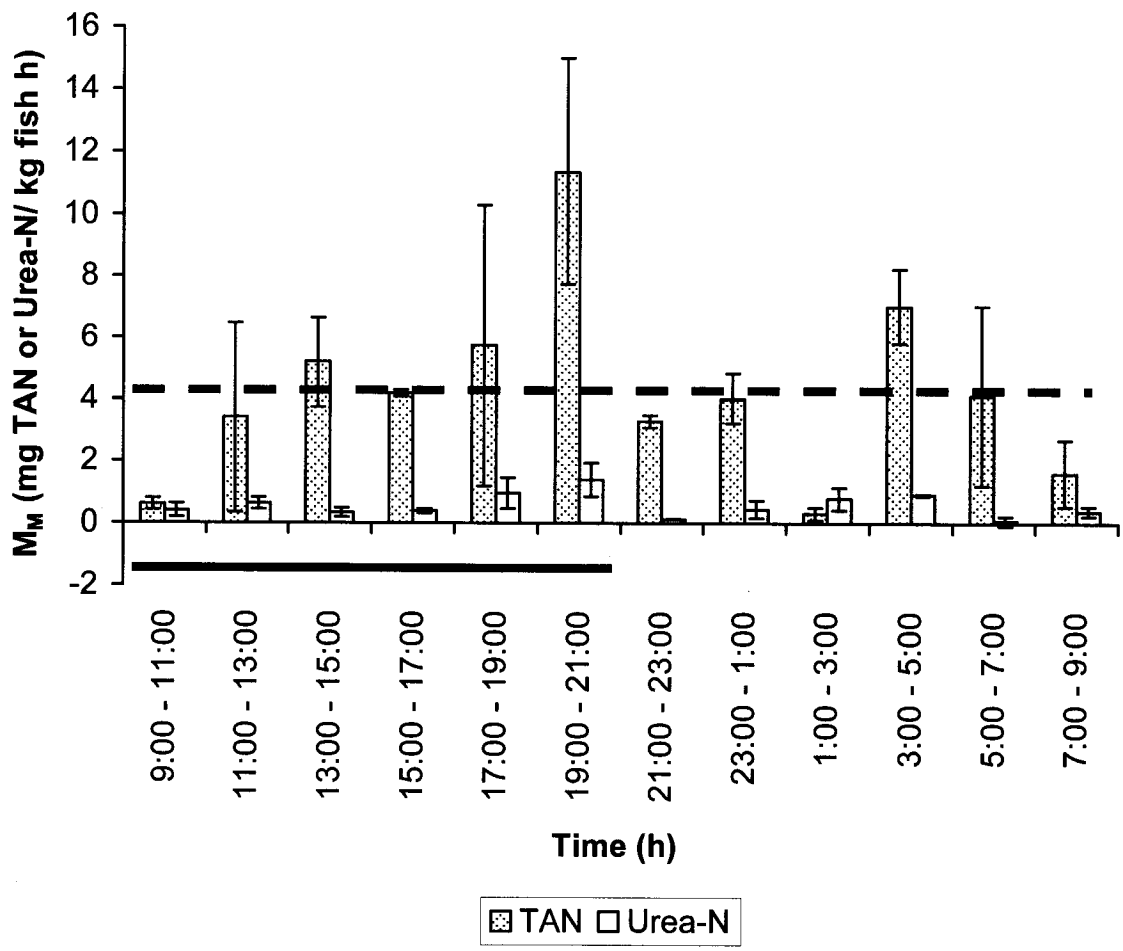

Figure 7.7.- Hourly TAN and urea-N excretion profile for 20 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

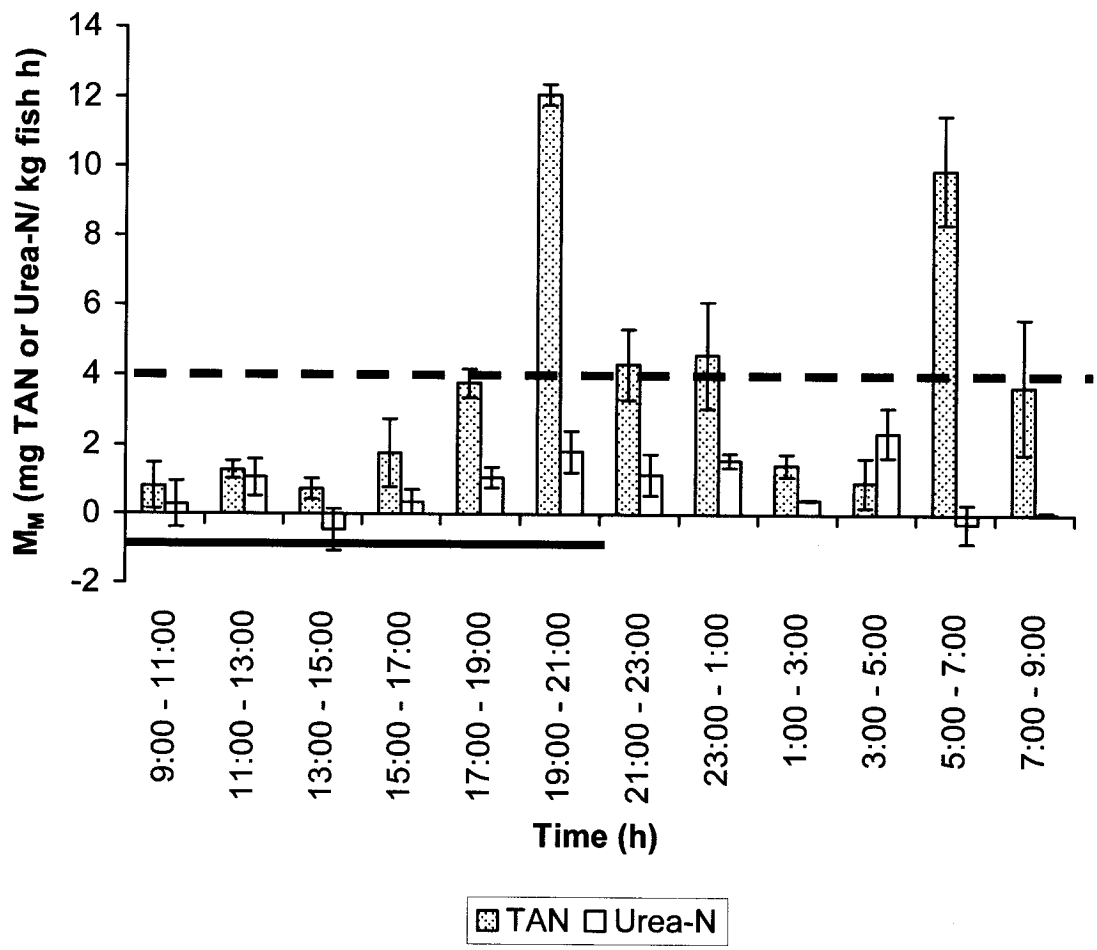

Figure 7.8.- Hourly TAN and urea-N excretion profile for 112 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

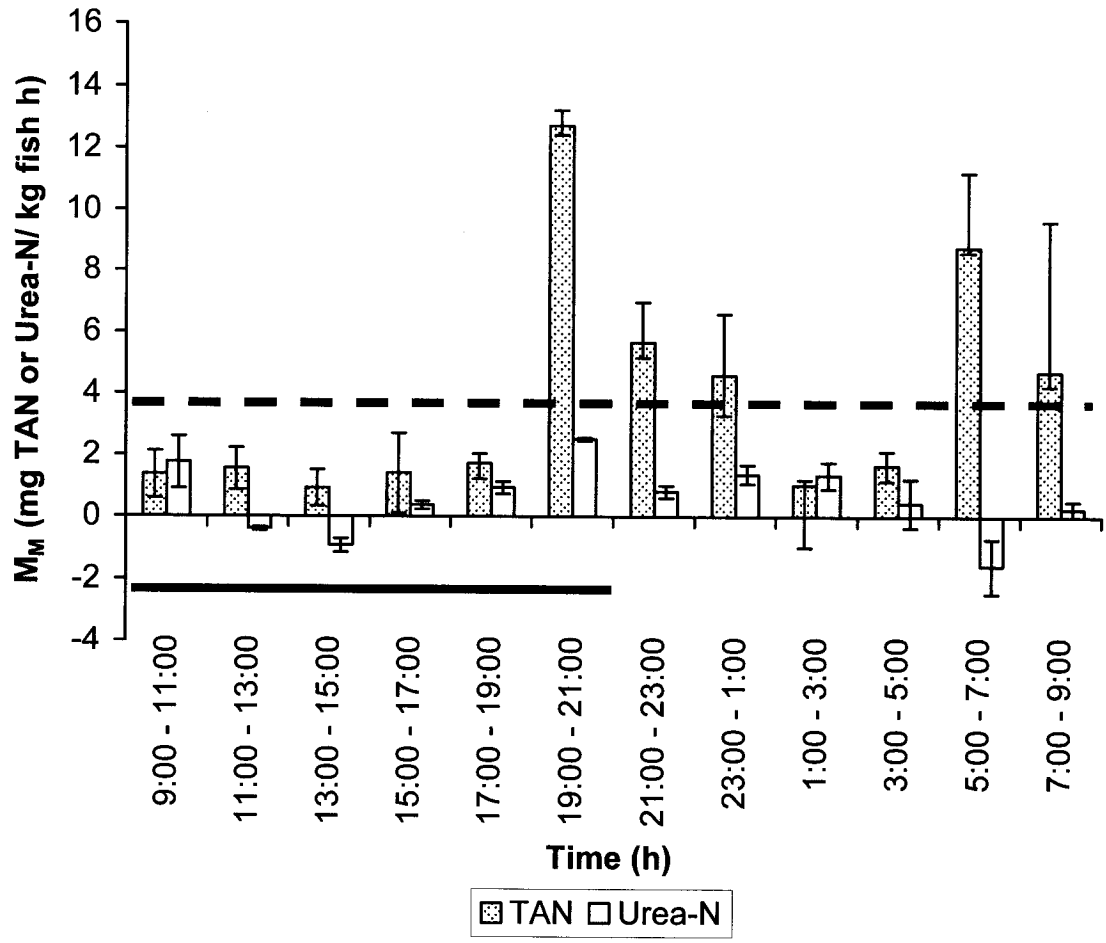

Figure 7.9.- Hourly TAN and urea-N excretion profile for 199 g juvenile California halibut. Feed was distributed continuously between 9:00 and 21:00 h during the light period. The black bar represents the feeding period. The dashed line shows the mean TAN excretion rate for the day.

TAN excretion rates showed some negative values for 7 g fish (Fig. 7.5) and for 199 g fish (Fig. 7.9). Urea-N excretion rates showed some negative values for 4 g fish (Fig. 7.4), 7 g fish (Fig. 7.5), 10 g fish (Fig. 7.6), 112 g fish (Fig 7.8), and for 199 g fish (Fig. 7.9).

For 4 and 7 g fish there were three peaks for TAN and one peak for urea excretion rate. TAN hourly excretion rates for hours 13:00-15:00, 15:00-17:00, and 19:00-21:00 h were significantly ($p<0.05$) higher than the other TAN excretion rates measured (Fig. 7.4 and Fig. 7.5). The variability in urea-N excretion rates was not as pronounced as TAN rates over a 24 h sampling period. Urea-N rate at 1:00-3:00 h was found to be significantly ($p<0.05$) higher than the other urea-N excretion rates measured (Fig. 7.4 and Fig. 7.5). Urea-N for 3 g fish was not significantly different ($p>0.05$) to hourly TAN excretion rates at times 9:00-11:00 h, 11:00-13:00 h, 17:00-19:00 h, and 23:00-1:00 h (Fig. 7.4). In addition, for 7 g fish urea-N was not significantly different ($p>0.05$) from TAN excretion rates at times 9:00-11:00 h, 17:00-19:00 h, 23:00-1:00 h, 3:00-5:00 h, and 7:00-9:00 h (Fig. 7.5).

For 10 and 20 g fish there were no peaks for TAN and two to three peaks for urea excretion rate. The smaller ($p<0.05$) TAN excretion rates happened at 9:00-11:00 h and 1:00-3:00 h (Fig. 7.6 and Fig. 7.7). Urea-N rates at 17:00-19:00, 1:00-3:00, and 9:00-11:00 h were found to be significantly ($p<0.05$) higher than the other urea-N rates measured for 10 g fish (Fig. 7.6) and at 21:00-23:00 and at 5:00-7:00 h for 20 g fish (Fig. 7.7). Urea-N was not significantly different

($p>0.05$) to hourly TAN excretion rates at times 9:00-11:00 h, 11:00-13:00 h, and 17:00-19:00 h for both fish sizes (Fig. 7.6 and Fig. 7.7), and also at 7:00-9:00 for 20 g fish (Fig. 7.7).

For 112 g fish there were two peaks for TAN and none for urea excretion rate. TAN excretion rates were significantly larger than at other times ($p<0.05$) between 19:00-21:00 h and 5:00-7:00 h (Fig. 7.8). Urea-N excretion rates were significantly lower ($p<0.05$) between 5:00-9:00 h and 13:00-15:00 h in comparison to the other urea-N excretion rates (Fig. 7.8). Urea-N excretion rates were similar ($p>0.05$) to TAN excretion rates at 9:00-11:00 h, 11:00-13:00 h, 15:00-17:00 h, and 3:00-5:00 h (Fig. 7.8).

For 199 g fish there were two peaks for TAN and three peaks for urea excretion rate. TAN excretion rates were significantly larger than at other times ($p<0.05$) between 19:00-21:00 h and 5:00-9:00 h (Fig. 7.9). Urea-N excretion rates were significantly larger ($p<0.05$) between 19:00-21:00 h and 23:00-3:00 h and 9:00-11:00 h in comparison to the other urea-N excretion rates (Fig. 7.9). Urea-N excretion rates were similar ($p>0.05$) to TAN excretion rates at times 9:00-11:00 h, 15:00-17:00 h, 17:00-19:00 h, 1:00-3:00 h, and 3:00-5:00 h (Fig. 7.9).

7.3.3.- TAN and urea-N excretion per unit of feed

TAN and urea-N excretion per unit of feed or per unit of N in the feed showed significant differences ($p < 0.05$) among the mean fish masses (Table 7.6). TAN excretion rates per unit of feed were similar between 4 and 7 g fish, but smaller

Table 7.6.- TAN and urea-N excretion rates and ratios. Similar letters following a number in an excretion rate row indicate no significant difference ($p > 0.05$). Number of replicates is two.

	Fish sizes					
Fish number	889	518	973	442	80	45
Biomass (g)	3708	3722	10113	8896	8933	8933
W (g)	4.2	7.2	10.4	20.1	111.7	198.5
PCA	245	198	466	316	205.28	170.76
%BW	2.1	2.4	1.2	1.2	1.05	1.05
Type of feed	SC 1	SC 2	EWOS 2	EWOS 2	EWOS 5	EWOS 5
% protein in feed	45	45	43	43	43	43
g feed/kg fish d	21	24	12	12	11	11
g N-feed/kg fish d	1.5	1.7	0.8	0.8	0.7	0.7
mg TAN/ g feed	4.9±0.5a	4.3±0.6a	8.5±1.2b	7.8±1.0b	7.9±0.4b	7.9±0.5b
mg Urea-N/g feed	0.8±0.1a	0.9±0.1a	1.1±0.1a	1.0±0.2a	1.8±0.1b	1.4±0.1b
Total N / g feed	5.7	5.8	9.6	8.8	9.7	9.3
mg TAN/g N-feed	67.4±7.4a	59.4±8.9a	123.8±18.1b	114.0±14.0b	115.1±6.0b	114.4±7.3b
mg Urea-N/g N-feed	11.0±1.4a	11.9±1.2a	16.7±2.0a	14.9±2.4a	26.0±1.5b	18.3±0.3b
Total N / g N-feed	78.4	71.3	140.5	128.9	141.1	132.7

than for the other fish. Fish weighing 10, 20, 112, and 199 g showed a similar TAN excretion per unit of feed. Urea-N excretion rates per unit of feed were similar among 4, 7, 10, and 20 g fish, but significantly smaller ($p<0.05$) than for 112 and 199 g fish (Table 7.6).

7.3.4.- Daily TAN and urea-N excretion per unit of fish mass

Daily TAN and urea-N excretion per unit of mass showed no significant differences ($p > 0.05$) among the mean fish masses (Table 7.7). The daily TAN excretion rate ranged from 82.7 to 102.7 mg TAN / kg fish d with a decreasing trend as fish size increased. The daily urea-N excretion rate ranged from 12.3 to 20.5 mg urea-N / kg fish d. The average percentage of daily urea-N excretion with respect to TAN was 17.1 ± 3.5%. Contribution of TAN and urea-N to total daily TAN plus urea-N excreted per unit of fish mass was 85.5 ± 2.5% and 14.5 ± 2.5%, respectively (Table 7.7).

7.3.5.- Daily and maximum hourly TAN and urea-N excretion rate per fish

Daily and hourly maximum TAN and urea-N excretion rates per fish unit for juvenile California halibut are shown in Fig. 7.10 and Fig. 7.11. Daily and hourly maximum TAN and urea-N excretion rates increased with fish mass on a per fish basis. For daily TAN and urea-N excretion rates, both power equations show a similar exponential value of ~0.96, which explains why the daily urea-N : TAN ratio remains constant with the increase in fish mass size (Fig. 7.10). Maximum hourly TAN and urea-N excretion rates showed a similar exponential value of

Table 7.7.- Daily TAN and urea-N excretion rates and ratios. There were no significant differences among fish groups ($p > 0.05$). SD = standard deviation. Number of replicates was two.

	Fish average mass (g)											
	4.2		7.2		10.4		20.1		111.7		198.5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
mg TAN/kg fish d	101.9	11.1	102.7	15.3	102.2	14.9	94.1	11.6	83.2	4.3	82.7	5.3
mg Urea-N/kg fish d	16.7	2.2	20.5	2.1	13.8	1.6	12.3	2.0	18.8	1.1	13.2	0.2
Urea-N / TAN, (%)	16.8	4.0	20.7	5.2	13.6	0.4	13.0	0.5	22.6	0.1	16.0	0.8
Urea-N / (Urea-N + TAN), (%)	14.3	2.9	17.0	3.5	11.9	0.3	11.5	0.4	18.4	0.1	13.8	0.6
TAN / (Urea-N + TAN), (%)	85.7	2.9	83.0	3.5	88.1	0.3	88.5	0.4	81.6	0.1	86.2	0.6

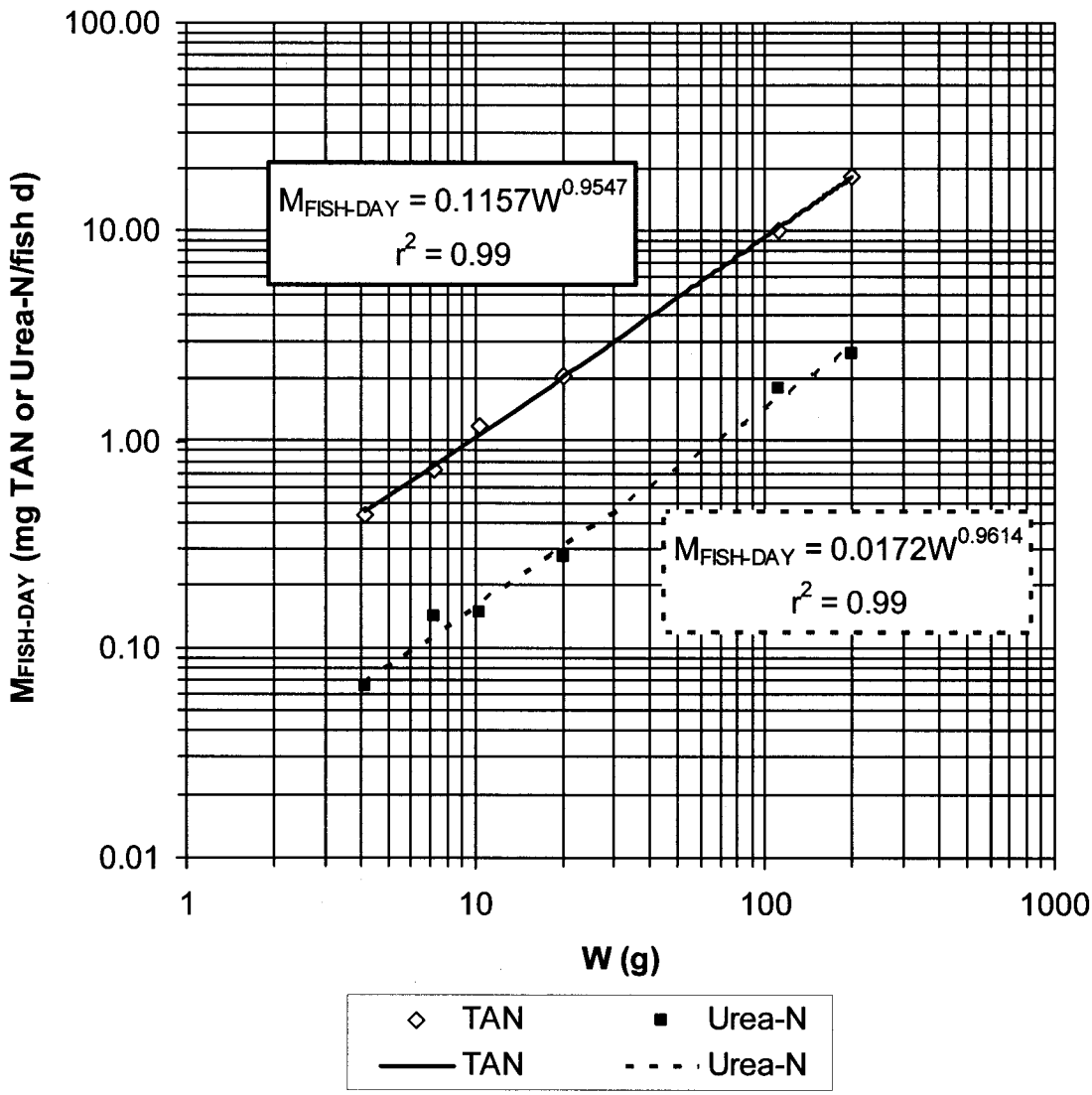

Figure 7.10.- Daily TAN and urea-N excretion per fish of juvenile California halibut.

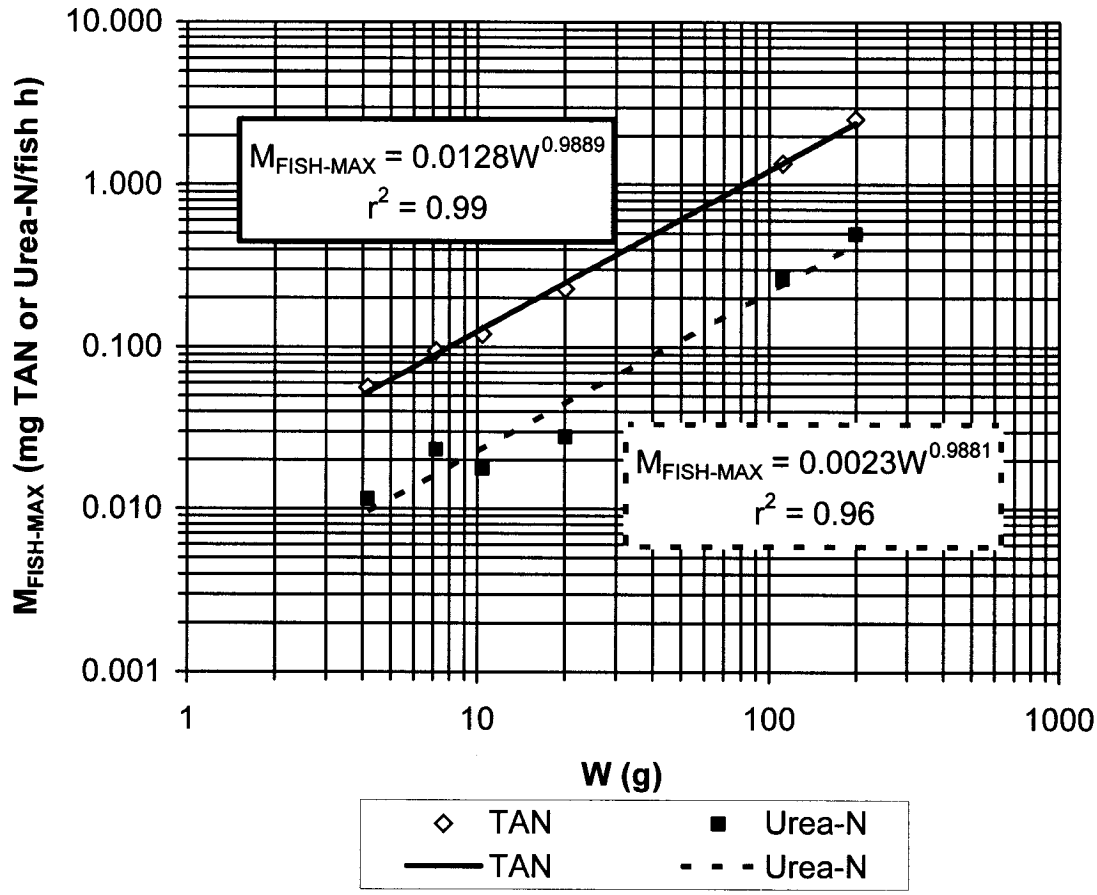

Figure 7.11.- Maximum hourly TAN and urea-N excretion per fish of juvenile California halibut.

~0.99, which explains why the maximum hourly urea-N : TAN ratio remains constant along with the increase in fish mass size (Fig. 7.11).

7.4.- DISCUSSION

The methodology followed in this research was very similar to that used by Thomas and Piedrahita (1998). As in their work, TAN and urea-N excretion rates measured here did not discriminate between the fish and microbial organisms present in the culture tanks. Therefore the term "apparent excretion rate" should be attached each time TAN or urea-N excretion for California halibut is referred to in this discussion. Furthermore, this research constitutes the first report on TAN and urea-N excretion rates for juvenile California halibut reared in a farm-like intensive marine recirculating system.

In general TAN levels measured (Table 7.5) were below the toxicity levels reported in the literature for other flatfish species (Alderson, 1979; Rasmussen and Korsgaard, 1996; Person-Le Ruyet et al., 1997). Therefore California halibut juveniles were not exposed to dangerous levels of TAN.

7.4.1.- Diel pattern of hourly TAN and urea-N excretion rates

The discussion will be presented below according to the type of culture vessel used for rearing California halibut juvenile. This classification is due to some feeding pattern differences observed in the fish reared within these two types of

vessels. Urea-N excretion rates for all fish sizes were similar to some TAN hourly excretion rates several times within a 24 h cycle. The comparison between hourly TAN and urea-N excretion rates observed in this research also will be discussed. Some negative values were observed for TAN and urea-N excretion rates. Hypotheses of these negative rates will be discussed.

7.4.1.1.- Raceways

The dark phase peak TAN for California halibut reared in the raceways (4 to 20 g fish) occurred between 3:00 and 7:00 h, and is probably related to the feed metabolism. Dosdat et al. (1995) said that depending of the ration level, postprandial TAN excretion in turbot can be extended up to 24 h after first feeding. In a study with juvenile Atlantic cod, peak excretion occurred 6.5 – 27 hours after feeding, depending on ration size and feeding frequency (Ramnarine et al., 1987).

The other two TAN peaks for fish reared in the raceways occurred during the feeding period between 13:00 and 15:00 h and the last one between 19:00-21:00 h, almost at the end of the 12 h continuous feeding period. The second peak was smaller in magnitude than the third apparent TAN excretion peak. Hirame fed once a day showed a TAN excretion rate immediately after feeding that was four times that of starved fish (Kikuchi et al., 1991). Turbot (10:00 and 16:00 h) and rainbow trout (8:00 and 17:00 h) fed twice a day had two TAN excretion peaks 6 h after being fed (Dosdat et al., 1995; Bergero et al., 2001). For greenback

flounder, peak TAN excretion occurred 3 h after feeding, and was lower for morning than for evening fed fish (Verbeeten et al., 1999).

Hourly diel patterns for urea-N excretion rates per unit of mass observed in this study of juvenile California halibut reared in raceways presented 0, 1 and 3 peaks of the same magnitude. Fish weighing 20 g statistically did not present peaks for urea-N excretion. A common peak time for to 10 g fish was at time 1:00-3:00 h. Two more peaks were observed for 10 g fish during the feeding time (light period) at times 9:00-11:00 h and 17:00-19:00 h. However, in the present work juvenile California halibut were fed continuously between 9:00 and 21:00 h and even though some hourly urea-N excretion peaks were found, urea-N excretion rate was relatively constant throughout the day.

Although urea synthesis and excretion is poorly understood, studies have indicated that urea excretion by flatfish is related to feed consumption (Kikuchi et al., 1991; Dosdat et al., 1995; Verbeeten et al., 1999). Kikuchi et al. (1991) said that for juvenile hirame fed a single ration (0.5, 1.0, and 1.5% BW) the rates of urea-N excretion were almost constant during the first 6 h, however peaks were observed at times 6-12 h, 12-24 h, and 24-48 h after feeding. Dosdat et al. (1995) reported that juvenile turbot fed twice a day showed a large single peak in urea excretion which appeared to be meal size dependant and occurred about 6 h after the first feeding. Verbeeten et al. (1999) stated that urea excretion by greenback flounder increased directly with ration and peaks were observed at 3

and 12 h after feeding. The relationship between feed consumption and urea excretion is not limited to flatfish, as Engin and Carter (2001), studying the urea-N excretion in Australian eel (*Anguilla australis australis*), found that urea-N hourly excretion rates were slightly higher 4 hours after each feeding.

7.4.1.2.- Circular tanks

Fish reared in the circular tanks showed two hourly peaks for TAN excretion, one during the light phase and the other during the dark phase. The light phase TAN peak occurred for 112 and 199 g fish during the feeding period at 19:00-21:00 h. The dark phase peak occurred at 5:00-7:00 h. Kikuchi found that the duration of the increased rate of TAN excretion in hirame (163 to 575 g) continued to more than 36 h when they were fed once at 1.5% BW. The two TAN peaks were of similar magnitude.

California halibut juveniles weighing 112 and 199 g were reared in circular tanks and fed continuously during the light phase with a 12 h belt feeder (9:00-21:00 h). The belt feeders were located nearest to the influent water, which allowed a good distribution of the pellets in the tank. California halibut juveniles reared in circular tanks showed two hourly TAN peak excretion rates, similar to peaks reported for fish that have been fed twice a day (Kaushik, 1980; Ramnarine et al., 1987; Dosdat et al., 1995). The two hourly peak TAN excretion rates calculated happened 10 h after the beginning and about 8 h after conclusion of the feeding.

Hourly diel patterns for urea-N excretion rates per unit of mass observed in this study of juvenile California halibut reared in circular tanks presented zero and three peaks. Fish weighing 112 g did not present peaks for urea-N excretion. Fish weighing 199 g had a peak during the dark period between 23:00 and 3:00 h, and two more peaks during the feeding time (light period) at 9:00-11:00 h and 19:00-21:00 h. Kikuchi et al. (1991) reported that juvenile hirame fed a single ration (0.5, 1.0, and 1.5% BW) had urea excretion peak rates at 6-12 h, 12-24 h, and 24-48 h after feeding. Verbeeten et al. (1999) reported that urea excretion peaks by greenback flounder were observed at 3 and 12 h after feeding. Urea-N excretion rates by 199 g fish resembles those of fish fed once or twice a day (Kikuchi et al., 1991; Verbeeten et al., 1999).

7.4.1.3.- Relationship between TAN and urea-N excretion rates

Flatfish have unique physiological characteristics in comparison to other teleost fishes, particularly in the importance of urea-N excretion (Kikuchi et al., 1992; Carter et al., 1998; Verbeeten et al., 1999). Although TAN and urea-N excretion rates have been studied for flatfish species, most comparisons have been focused on the overall daily nitrogen budget (Kikuchi et al, 1991; Dosdat et al., 1995; Verbeeten et al., 1999). As discussed above, the application of feed to a rearing system initiates a process of metabolism and assimilation. This process occurs not only in the target fish population, but for the rearing system as well. When operating a recirculating aquaculture system, the rearing system itself must deal with the metabolic load through water treatment processes.

Quantification of TAN excretion is important for estimating maximum stocking biomass/density, water flow and the size of biological filters in culture systems (Paulson, 1980; Porter et al., 1987; Forsberg and Summerfelt, 1992; Wu, 1995; Timmons et al., 2001).

In the present research hourly TAN and urea-N excretion rates had similar magnitudes several times a day. California halibut juveniles also had occasional hourly urea-N excretion rate larger than hourly TAN excretion rate. Although not discussed by the authors (Kikuchi et al., 1991; Dosdat et al., 1995; Verbeeten et al., 1999), similar observations regarding the relationship between hourly TAN and urea-N excretion rates by hirame, turbot, and greenback flounder can be observed from their data.

Urea-N can be hydrolyzed rapidly to TAN in culture systems if urea-hydrolizing bacteria are present (Colt and Armstrong, 1981; Pedersen et al., 1993). Therefore, TAN produced from the urea-N hydrolysis will be part of the overall TAN budget in the culture system (Kikuchi, 1995). It is important then to determine the combined TAN plus urea-N values to use as design benchmarks. High TAN concentrations might reduce growth or cause mortalities in the target fish reared. In addition too high and low TAN concentrations might affect the biofilter performance, in the first case by inhibiting the *Nitrobacter sp.* bacteria (Horowitz and Horowitz, 1997), and in the latter by reducing the TAN availability for *Nitrosomonas sp.* bacteria (Brune and Gunther, 1981; Guger and Boller,

1986; Kugaprasatham et al., 1991; Kim et al., 1997). Consequently, the magnitudes of the hourly TAN and urea-N excretion rates as a whole are important not only for the fish health but also for an adequate biofilter operation.

7.4.1.4.- TAN and urea-N negative excretion rates

Horowitz and Horowitz (1997) studying the nitrification process in a Tilapia recirculating system stated that nitrification took place in microbially coated solid support medium, but fish water alone had a negligible TAN removal activity. Therefore, a possible explanation of negative TAN excretion rates, which happened both for 7 and 199 g California halibut juveniles, could have been due to nitrification by nitrifiers attached to the tank walls.

Urea-N is rapidly hydrolyzed to TAN if urea-hydrolyzing bacteria are present (Colt and Armstrong, 1981; Pedersen et al., 1993). Ureolytic bacteria have been reported to be present in animal feces (Chenost and Kayouli, 1997). Therefore, the presence of ureolytic bacteria might have been the reason for having negative urea-N excretion rates in this research. A small test performed in this research (data no presented), to the last water samples taken during a TAN-urea excretion trial, yielded no urea-N hydrolysis into TAN after 8 h of incubation. Microbial ureases, which are the enzyme that hydrolyzes urea-N into TAN, are primarily recognized as intracellular enzymes (Stocks-Fisher et al., 1999). In addition, ureolytic bacteria have been reported to grow mainly on surfaces ((Hammes et al., 2003; Udert et al., 2003). Consequently, it is likely that urea-

hydrolyzing bacteria were not suspended in the water which would explain why urea-N was not hydrolyzed to TAN in the water samples, but attached to the tank walls and within fish feces. Therefore some negative values for urea-N excretion rates could have been due to presence of urea-hydrolizing bacteria on the surfaces within the test vessels.

7.4.2.- TAN and urea-N excretion rates per unit of feed

Greenback flounder TAN and urea-N excretions were significantly affected by ration and feeding time and were larger in the evening and increased with an increase of the feed ration (Verbeeten et al., 1999). TAN excretion per unit of feed offered to California halibut shows two groups: one group with a low excretion rate (4 and 7 g fish with 4.9 and 4.3 mg TAN / g feed), and another with a larger excretion rate (10 to 199 g fish with 7.8 to 8.5 mg TAN / g feed). These two groups were fed with feeds having different nutritional compositions: Silver Cup® and Ewos®.

It has been shown in several freshwater and marine species that TAN excretion is proportional to the nitrogen content in the feed given (Haskell, 1955; Rychly, 1980; Meade, 1985; Brunty et al., 1997). In the present work, assuming that all feed given was eaten, California halibut fed with Silver Cup® ate between 1.5 and 1.7 g N / kg fish per day while those fed with Ewos® eat between 0.7 and 0.8 g N / kg fish per day (Table 7.6). Consequently, more TAN should have been excreted by California halibut juveniles fed with Silver Cup® than with EWOS®.

However, the low excretion group was fed with Silver Cup®, and the higher excretion group with Ewos®. Recent studies in California halibut (Bush, 2003) have shown that, for feeds containing a constant protein composition, a higher TAN excretion rate should be expected when the fat composition is low. Bush's (2003) observations and the results from this research agree with the literature (Ballestrazzi et al., 1994; Kolsater, 1995; Dias et al., 1998; Kaushik, 1998). The largest nitrogenous excretion found for California halibut were observed for the fish fed with EWOS™, particularly in the form of mg TAN / g N-feed, which were about twice of to those fed with Silver Cup™ (Table 7.6). Therefore nitrogenous excretion by fish can be reduced by decreasing protein levels (dietary digestible protein) and increasing fat levels (digestible energy), while keeping constant the total energy in the feed (Ballestrazzi et al., 1994; Kolsater, 1995; Thorpe and Cho, 1995).

As in the case of TAN, there were two groups according to urea-N excretion: one group with a low excretion rate (4 to 20 g fish with 0.8 to 1.1 mg urea-N / g feed), and another with a higher excretion rate (112 and 199 g fish with 1.4 to 1.8 mg urea-N / g feed). It has been found that urea-N excretion in plaice, hirame, turbot, and greenback is linked to the amount of digestible nitrogen administered (Jobling, 1981; Kikuchi, 1995; Dosdat et al., 1995, 1996; Verbeeten et al., 1999). However, the differences noticed in urea-N excretion in this research for California halibut may reflect the overall metabolism of the fish rather than the

effect of digestible nitrogen administered. In the present work differences in urea-N excretion were almost non-existent relative to feed or N-feed (Table 7.6).

Farmers should aim to provide sufficient protein nitrogen within the diet to promote growth (Carter and Bransden, 2001). The size of the ration also depends on the energy content of the food (Grove et al., 1985). Protein within the fish feed is the source of TAN introduced into production systems and higher levels of protein should result in higher levels of TAN production (Brunty et al., 1997), which has been reported for some flatfish species (Kikuchi et al., 1991; Dosdat et al., 1995; Verbeeten et al., 1999; Carter and Bransden, 2001). Studies in teleost fish relating TAN levels to feed protein content report that 1 kg of feed will produce between 20 and 300 g TAN (Rychly, 1980; Meade, 1985; Mires and Amit, 1990; Wright, 1993; Brunty et al., 1997). Several studies in flatfish species have investigated the effect of feed ration on the quantity of TAN and urea-N excreted, but did not report the excretion levels in terms of feed or feed protein content (Kikuchi et al., 1991; Dosdat et al., 1995; Kikuchi, 1995; Kikuchi et al., 1995; Dosdat et al., 1996; Verbeeten et al., 1999; Carter and Bransden, 2001). However, the nitrogen-feed relationship for flatfish was estimated from author's data to be between 0.5 and 27.6 g TAN / kg feed and between 0.12 and 38.09 g urea-N / kg feed, for feeds having a protein content between 47 and 55.2% (Table 7.1). Nitrogen excretion levels observed in this research by California halibut juveniles ranged from 4.9 to 8.5 g TAN / kg feed and from 0.8 to 1.6 g urea-N / kg feed, for feeds having a protein composition between 43 and 45%. California halibut juveniles excretion rates by unit of feed determined in this

research are within the range reported for other flatfish species as described above and are substantially lower than for other teleosts.

7.4.3.- TAN and urea-N excretion rate per unit of fish mass

Marine teleosts have basal nitrogen excretion rates between 1.4 and 34.9 mg TAN / kg h, and feeding can increase the excretion rate to 600 mg TAN / kg h in some fish species (for a review, see Handy and Poxton, 1993). Average hourly TAN and urea-N excretion rates can be calculated from the daily values (Table 7.7) and the TAN values are shown in Fig. 7.4 to7.9.

Fed California halibut juveniles showed an hourly TAN excretion rate between 3.8 and 4.7 mg TAN / kg fish h (Fig. 7.4 to Fig. 7.9). This is consistent with mean rates of output of 5.1 mg TAN / kg fish h for fed Atlantic halibut (Davenport et al., 1990), 2.7 to 30 mg TAN / kg h for flounder (Jobling, 1981), 2.9 to 10.2 mg TAN / kg h for turbot (Burel et al., 1996; Dosdat et al., 1995; Dosdat et al., 1996), and 5.4 to 21.9 mg TAN / kg h for hirame (Kikuchi et al., 1991; Kikuchi et al., 1995; Kikuchi, 1995).

Hourly urea-N excretion rates for California halibut were between 0.5 and 0.8 mg urea-N / kg fish h, which are similar to those reported for other flatfish, such as turbot which had excretion rates between 0.7 and 3.3 mg urea-N / kg fish h (Dosdat et al., 1995; Dosdat et al., 1996) and hirame with excretion rates between 0.7 and 2.8 mg urea-N / kg fish h. Greenback flounder were the flatfish

species with the largest urea-N excretion reported: 2 -16 mg urea-N / kg fish h (Verbeeten et al., 1999).

In this study, juvenile California halibut cultured under farm-like conditions, had daily TAN and urea-N excretion rates per unit of fish mass that did not differ between the fish masses tested, regardless of fish size and diet. Other studies on flatfish have reported a decrease in TAN and urea-N excretion rates per unit of fish mass for larger fish in comparison to smaller fish (Kikuchi et al., 1990; Kikuchi, 1995; Dosdat et al., 1996). However, those studies fed the animals once (morning) or twice (morning and evening) to satiation or with a larger feed ration, in comparison to the rations given here to California halibut juveniles.

TAN is the main nitrogenous excretion product from fish and typically constitutes 80 to 90% of the total nitrogen excreted (Fivelstad et al., 1990; Handy and Poxton, 1993). The overall results from this study showed that 86 ± 3% of the daily nitrogen excreted (TAN plus urea-N) corresponded to TAN excretion. Similar values have been reported for other flatfish species, with 75 to 85% for plaice (Jobling, 1981), 78 to 83% for hirame (Kikuchi et al., 1990; Kikuchi, 1995; Kikuchi et al., 1995; Tanaka and Kadowaki, 1995), 78 to 82% for turbot (Dosdat et al., 1996), and with 37 to 88% for greenback flounder (Verbeeten et al., 1999). After TAN, urea-N is the next form of nitrogenous excretion, which is typically reported to account for under 20% of the total nitrogen excretion in teleost fish (Colt and Armstrong, 1981; Chadwick and Wright, 1999; Kajimura et al., 2002). In this study, 15 ± 3% of the nitrogen excreted (TAN plus urea-N) corresponded to

urea-N, which was similar to those reported for starry flounder with 12% (Wood, 1958), for hirame with 17 to 22% (Kikuchi et al., 1991; Kikuchi, 1995; Tanaka and Kadowaki, 1995), for turbot with 18 to 22% (Dosdat et al., 1995), and for greenback flounder with 12 to 63% (Verbeeten et al., 1999; Carter and Bransden, 2001).

7.4.4.- TAN and urea-N excretion rate per fish

Smaller fish excreted less TAN and urea-N than larger fish, a fact that has been reported for hirame (Kikuchi, 1995; Tanaka and Kadowaki, 1995). The maximum nitrogen excretion rate per fish might be used as a design tool for sizing and management a recirculating aquacultural system for California halibut. Further studies are needed to model nitrogen excretion by California halibut under other rearing conditions, feed composition, and ration.

The juvenile California halibut studied here were kept at high densities ranging from 171 to 466% PCA. There are no studies in flatfish species reporting the effect of density on TAN and urea-N excretion rates. However a study done with rainbow trout found that higher biomass did not lower the TAN excretion on a per fish basis (Wagner et al., 1995). The effect of California halibut stocking density on TAN and urea-N excretion rate was not explored in this research.

7.5.- CONCLUSSIONS

This research constitutes the first report on TAN and urea-N excretion rates for juvenile California halibut reared in a farm-like intensive marine recirculating system. The main conclussions were:

a) This study showed that there were peaks for TAN and urea-N excretion when juvenile California halibut were fed continuously over 12 hours during the light phase period, regardless of the fish size and tank shape. Hence, if a recirculating system for California halibut is designed to operate as the one described in this research, then a routine TAN and urea-N concentrations should be monitored prior to feeding at 9:00 h and between 19:00 and 21:00 h, when peak TAN excretion rates and concentrations in the system are likely to take place.

b) Low nitrogenous excretion rates relative to feed, feed N, and fish mass in juvenile California halibut were observed in this research compared to other teleosts, which is an advantage in the design of recirculation systems for their cultures. TAN excretion due to feed was between 4.3 and 8.5 mg TAN / g feed. Urea-N excretion due to feed was between 0.8 and 1.8 mg urea-N / g feed. TAN excretion due to feed N was between 59 and 124 mg TAN / g N feed. Urea-N excretion due to feed N was between 11 and 26 mg urea-N / g N feed. Daily TAN excretion was between 91

and 113 mg TAN / kg fish d. Daily urea-N excretion was between 13 and 20 mg urea-N / kg fish d.

c) Although urea-N makes up a relatively small portion of the total daily nitrogen excretion (urea-N plus TAN) by juvenile California halibut, there were times during the day during when urea-N excretion rate was similar to or larger than TAN excretion rates.

d) TAN excretion accounted for 84 to 89% of the total daily urea-N plus TAN excretion by juvenile California halibut. Urea-N excretion accounted for 11 to 16% of the total daily urea-N plus TAN excretion by juvenile California halibut.

e) TAN excretion was between 4.3 and 8.5 g TAN / kg feed. Urea-N excretion was between 0.8 and 1.8 g urea-N / kg feed. There is an apparent impact of energy content (feed composition) on the amount of TAN excreted. It has been reported that a higher fat content in the diet relative to protein will diminish the TAN excreted by California halibut (Bush, 2003).

f) The per fish TAN and urea-N excretion rates of juvenile California halibut increased with fish size.

7.6.- FURTHER RESEARCH

Further studies are recommended in the following areas:

- Determine the effect of feeding regime, ration and feeding time on TAN and urea-N excretion.

- Although this study did not reveal an effect of stocking density on TAN and urea-N excretion, this observation should be confirmed.

- Determine if California halibut can switch from TAN to urea synthesis if the surrounding environment reaches a TAN threshold limit. And under this condition determine the growth reduction due to energy spent for urea synthesis.

- Since low nitrogenous excretion rates for juvenile California halibut were observed in this study, it is recommended that studies be pursued to determine the efficiency of the use of dietary proteins versus nitrogen excretion rates and fish growth.

CHAPTER VIII

SETTLING VELOCITY OF SOLIDS SETTLED WITHIN CALIFORNIA HALIBUT RACEWAYS

8.1.- INTRODUCTION

Wastes from aquaculture include all materials used in the process that are not removed from the system during harvesting (Cripps and Bergheim, 2000). The main particulate wastes from marine recirculating finfish systems are feces, uneaten feed, and fish mucus (Chen et al., 1993b; Patterson and Watts, 2003). It has been reported for salmonids that 1 kg of feed produces about 0.3 to 0.4 kg of fecal solids (Smith et al., 1980; Beveridge et al., 1991; Bergheim and Brinker, 2003; Patterson and Watts, 2003) and for catfish about 0.2 to 0.7 kg of fecal solids (Chen et al., 1997). Other sources of particles may be filters that release some of the filter media or bacterial flocks to the recirculated water. These suspended particles will vary greatly in size, from cm to μm size (Timmons et al., 2001; Patterson and Watts, 2003).

A buildup of solids in a recirculating aquaculture system can lead to problems with the recirculating system components (Wheaton, 1977; McMillan et al., 2003) and fish performance (Larmoyeux and Piper, 1973; McConnell, 1989; Noble and Summerfelt; 1996). These detrimental effects can be worse if the particles are

not removed as they are generated within the system. Particle sizes can be reduced by mechanical action, such as pumping, resulting in particles that are more difficult to remove (McMillan et al., 2003).

The detrimental effects of suspended solids (SS) on recirculating system components include clogging of biological filters, reduction of the effectiveness of ultra-violet water treatment, increased need of backwash of mechanical filters, increased heterotrophic bacteria within biofilters, increased biochemical oxygen demand, and mineralization that produces TAN (Wyban and Sweeny, 1989; Chen and Malone, 1991; Chen et al., 1993b; Cripps and Bergheim, 2000; Sumagaysay-Chavoso and San Diego-McGlone, 2003; Patterson and Watts, 2003).

Suspended solids can cause a decline in water quality (Chen et al., 1993a, 1993b) that will create a variety of problems including stress on the culture organisms (Wedemeyer, 1996; Poxton and Allouse, 1982). Large quantities of SS may suffocate developing eggs during incubation or physically abrade or coat the gills of fish (Wedemeyer, 1996). Turbidity due to SS may interfere with sight feeding fish species such as plaice (Batty and Hoyt, 1995) and turbot (Mallekh et al., 1998) at SS above 65 mg/L. Turbidity and SS levels favoring optimum fish health have not been determined (Wedemeyer, 1996; Timmons et al., 2001).

Although there is concern about the presence of suspended solids in aquacultural systems (Liao, 1981; Cripps, 1994; Twarowska et al., 1997; Bergheim et al., 1998), little work has been done to quantify the physical characteristics of solids particles. Clark et al. (1985) found that the shear resistance of suspended particles from trout wastewater was lower than for sewage flocs. Chen et al. (1993 a, b), Cripps (1995) and Kelly et al. (1997) described the suspended solids from salmon farms in terms of their nutritional composition and size distribution. Chen et al. (1999 a, b) and Wong and Piedrahita (2000) studied particle mass settling velocity distribution associated with suspended solids taken from a salmonid farm. Patterson and Watts (2003) and Patterson et al. (2003) determined the density and size of particles from a salmonid farm. Overall, research on suspended solids has occurred mainly with salmonid species, and to the author's knowledge this is the first study performed with a flatfish species.

After metamorphosis, flatfish leave the water column and start living on the tank bottom. The settling of particles within a flatfish culture tank can be especially problematic given the fish's behavior. Hence there is a need to determine settling data for solids that may be present within California halibut culture vessels. The information can be used to develop tanks for flatfish culture in which solids do not settle, as well as to determine the potential for the use of solids removal technologies as part of a water treatment system.

8.1.1.- Literature review

The following section will start with a brief description on how the solids are classified. This description will help to understand the terminology associated with solids classification. Thereafter, information available on aquacultural solids characteristics will be presented, particularly in terms of density, size and settling velocity. Finally, the type of information needed for designing the most common devices used for solids removal in aquaculture and their limitations will also be discussed briefly.

8.1.1.1.- Solids classification

Particulate materials in wastewater may be classified as settleable, suspended, dissolved, or colloidal (Metcalf & Eddy, Inc., 1991). Settleable solids are those that will settle in an Imhoff cone in one hour (APHA, 1995). Suspended and dissolved solids are differentiated through the use of a membrane filter having a pore size of about 1.2 μm (APHA, 1995; Lawson, 1995). Particles that are trapped on the filter are called suspended solids (SS).

8.1.1.2.- Density, size, and settling velocity of aquacultural particles

Attempts have been made in aquaculture to determine the densities of solids of feed origin. Chen et al. (1993b) reported an overall mean density of 1190 kg/m^3 (specific gravity of 1.19). An anonymous source (1995, cited by Patterson et al., 2003) found that, for a salmonid recirculating system, the density of fecal material

was 1050 kg/m^3. Patterson et al. (2003), studying the solids from more than ten fish farms found that particle density ranged from 1050 to 1153 kg/m^3.

Cripps (1995) found that the particle size distribution of untreated effluent from a salmonid hatchery was within the range of 8.4 to ~155 µm. McMillan et al. (2003) reported particle size distribution in a recirculating system as ranging from 0.4 to ~12 µm, with most of the particles concentrated from 0.4 to 2 µm. According to Chen et al. (1993b), 95% of the suspended particles in recirculating aquacultural systems are smaller than 20 µm and account for 40-70% of the total mass of SS.

Patterson and Watts (2003), studying solids from a commercial Atlantic salmon smolt production facility, found that there was a wide range of particle shapes from long spicule-like particles to sand-like particles. Furthermore, Patterson et al. (2003) found that particles of a given size and shape could have different densities.

Settling velocities of particulate material produced by aquaculture facilities have not been extensively studied, and there are only a few reports with such information. The reported mean settling velocity for settleable solids from rainbow trout was 1.7 cm/s and for manually stripped fecal material was 0.7 cm/s (Wong and Piedrahita, 2000); between 4 and 6 cm/s for fecal pellets from salmonids, (Gowen and Bradbury, 1987); and between 5.1 and 6.4 cm/s for fecal pellets

from Atlantic salmon (Chen et al., 2003). Feed pellets were found to have a faster settling velocity than feces, with reported values of 15 to 33 cm/s (Juell, 1991). Settling velocities for extruded diets for sea bream and sea bass were reported to range from 3.9 to 10.6 cm/s (Chen et al., 2000).

8.1.1.3.- Settling velocity measurement

During the 1980s, the search for better sewer designs for the treatment of municipal wastewater led to an interest in the measurements of the settling velocity characteristics of sewer solids *in situ* (Hedges et al., 1998) rather than through calculations based on measurements of particle size, density and shape (ie. using Stokes law) (Aiguier et al., 1996; Loch, 2001). The calculation approach to determining settling velocity is not recommended due to the large variation in particle size, shape, and specific gravity (Hedges et al., 1998; Patterson and Watts, 2003). Methods for determining settling velocity *in situ* are well documented elsewhere (Michelbach and Wohrle, 1992; Michelbach and Wohrle, 1993; Aiguier et al., 1996; Tyack et al., 1996; Wong and Piedrahita, 2000).

Settling velocity measurements use either dispersed or stratified systems. In a dispersed system, particles begin to settle from an initial uniform dispersion, whereas in a stratified system, the particles begin falling from a common source and become stratified according to settling velocities. Stratified systems are the most common method used to determine settling velocities (Gibbs et al., 1971;

Michelbach and Wohrle, 1992; Aiguier et al., 1996; Wong and Piedrahita, 2000; Loch, 2001). They are essentially tubes in which the sample is introduced at the top, and sediment settling to the bottom of the tube is sampled several times in some way. For a detailed comparison of the most common stratified methods used see Aiguier et al. (1996) and Lucas-Aiguier et al. (1998). The stratified system to be used in this research, known as the UFT method, has been described by Wong and Piedrahita (2000).

According to Aiguier et al. (1996), the settleable solids to be used for the UFT-method are collected by settling for 2 h in an Imhoff cone. Usually the mass of solids to be used for the stratified settling test is between 1 - 5 g/L of sample material depending on the volume of the settling column (Aiguier et al., 1996; Lin, 2001; Loch, 2001).

8.1.1.4.- Settling velocity distribution curves

In the design of sedimentation tanks, a particle terminal velocity, v_{sc} (critical settling velocity), is used as a design overflow settling velocity (Mihelcic, 1999). A term called overflow rate (OFR, m^3 / m^2 h) or surface loading rate can be defined as (Wong and Piedrahita, 2000):

$$OFR = v_{sc} = \frac{Q}{A_s} \qquad \text{(Eq. 8.1)}$$

where, Q = volumetric flow rate (m^3 / h)

A_s = surface area of sedimentation tank (m^2)

In theory, all particles in a sedimentation tank, regardless of entry point, having settling velocity $v_s \geq v_{sc}$ will be completely removed or settled. Some fraction of particles in a sedimentation tank having $v_s < v_{sc}$ will also be removed depending on entry location.

The data obtained from a settling velocity test can be used to construct a cumulative settling velocity distribution curve (Fig. 8.1). Typically, settling velocity distribution curves are produced as cumulative graphs which show the proportion of material by mass, with settling velocities less than or equal to a given settling velocity (Ando and Smisson, 1996; Wong and Piedrahita, 2000; Lin, 2001). The settling curve (Fig. 8.1) is organized so that the faster settling particles will be to the right and the slower settling particles will be to the left.

The theoretical overall fraction removal (η, %) for a sedimentation tank can be defined as (Wong and Piedrahita, 2000; Lin, 2001):

$$\eta = (1 - F_o) + \frac{1}{v_{sc}} \int_0^{F_o} v_s dF \qquad \text{(Eq. 8.2)}$$

where, F_o = mass fraction of particles with a $v_s < v_{sc}$

v_s = particle settling velocity (cm / s)

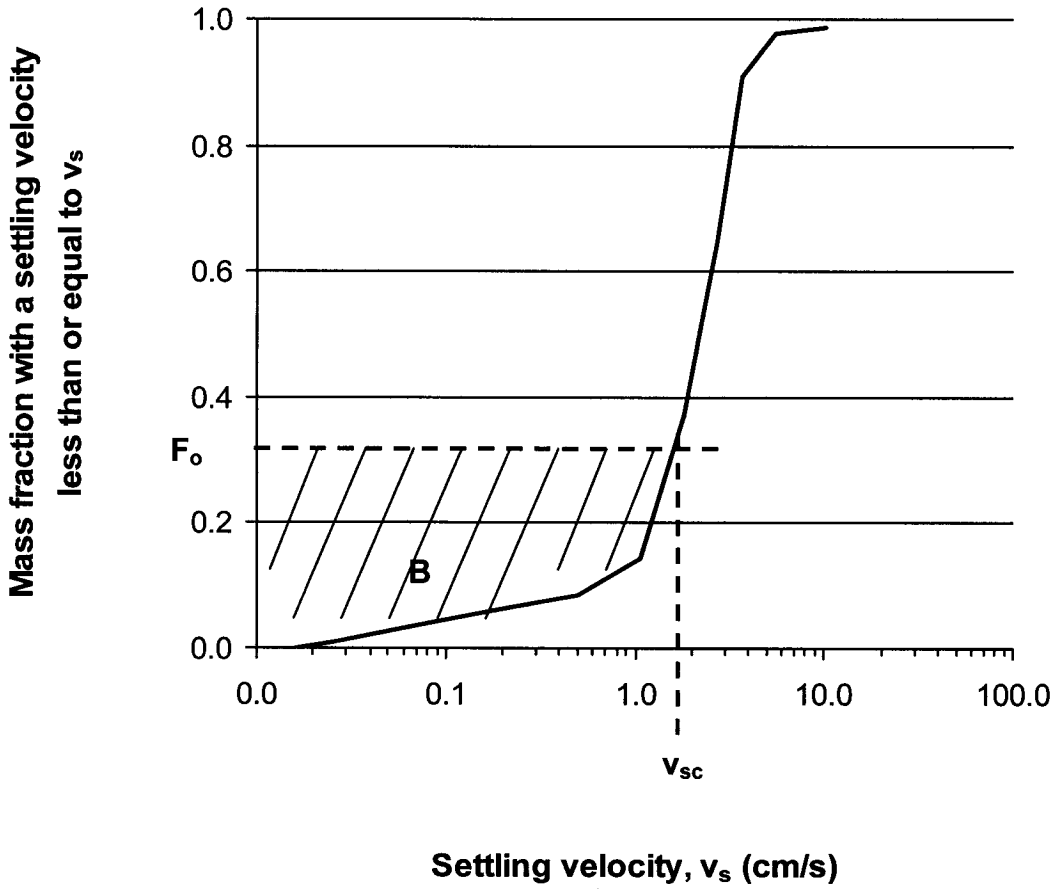

Figure 8.1.- Example of a cumulative settling velocity distribution curve. Terms are as defined in the text.

If the integral in the above expression is defined by B (area on the graph in Fig. 8.1), the equation can be rewritten as (Wong and Piedrahita, 2000; Lin, 2001):

$$\eta = (1-F_o) + \frac{B}{v_{sc}} = (1-F_o) + \frac{1}{v_{sc}} \sum v_s \Delta F \qquad \text{(Eq. 8.3)}$$

where the summation serves as an approximation of the integral in Eq. 8.2. A settling velocity distribution curve can be used to evaluate the changes in overall fraction removal by altering OFR for a given sedimentation tank. A non-linear equation describing the relationship between F_o and v_s was proposed by Wong and Piedrahita (2000) to facilitate the estimation of η using Eq. 8.2 or Eq. 8.3:

$$F_o = a + \frac{b}{(1+(\frac{v_s}{c})^d)} \qquad \text{(Eq. 8.4)}$$

where a, b, c, and d are parameters specific to a given settling velocity distribution.

8.1.1.5.- Determination of aquacultural particle sizes

Knowledge of the particle size distribution of aquaculture effluents is most relevant for the optimization of mechanical treatment attempts. For the characterization of aquacultural suspended particle sizes, two methods have been used: the fractionation of solids by the use of screens (Cripps, 1995; Patterson and Watts, 2003; Patterson et al., 2003), and the counting and sizing

of particles by the use of the Coulter® method (Cripps, 1995; McMillan et al., 2003).

Settling velocity distribution curves can be used to determine a relationship for settling velocity and particle diameter (Gibbs et al., 1971; Wong, 2002). The process for determining such a relationship requires having a particle with a known size which has the same density and settling velocity as the unknown particle under study (Isaacs and Thodos, 1967; Gibbs et al., 1971). As mentioned earlier in this review, the densities, sizes and shapes of solids for aquaculture varied widely (Chen et al., 1993; Patterson et al., 2003), which makes it difficult to define a unique expression that can relate particle size with settling velocity. Studies done by Chen et al. (1999, 2003) in salmonid wastewater reported that there was no clear relationship between fecal pellet mass and settling velocity; furthermore, fecal sizes proved to be a poor predictor of settling velocity. The findings of Patterson et al. (2003) and Chen et al. (1999, 2003) demonstrate that there are difficulties in the assignment of a representative equivalent particle size for a given settling velocity in the analysis of aquacultural solids. Therefore the settling velocity should not be used to estimate particle size given uncertainty about particle density and shape.

8.1.1.6.- Removal methods for aquacultural particles

There is extensive literature about waste and waste treatment that focuses on the design of devices to remove solids from domestic waters (Metcalf & Eddy,

Inc., 1991; Andoh and Smisson, 1996; Michelbach and Weib, 1996; Swamee and Tyagi, 1996; Crites and Tchobanoglous, 1998; Lin, 2001). Devices used to remove particulate matter from water utilize one of the five particle attributes: density, size or electrical, chemical, or magnetic properties. The aquaculture industry has adopted some of the technology used in municipal waste and water treatment plant into farm design and operation (Wheaton, 1977; Muir, 1982; Coll, 1991; Chen et al., 1992; Chen et al., 1993; Lybey, 1993; Lawson, 1995; White and Townsend, 1996; Twarowska et al., 1997; Hussenot et al., 1998; Timmons et al., 2001; Bergheim and Brinker, 2003; Ebeling et al., 2003; Schulz et al, 2003). The devices adopted by the aquaculture industry focus mainly on density and particle size to remove particles from the rearing water (Scott and Allard, 1984; Chen and Malone, 1991; Eikebrokk and Ulgenes, 1993; Libey, 1993; Lunde and Skybakmoen, 1993; White and Townsend, 1996; Kelly et al., 1997; Bergheim et al., 1998; Cripps and Bergheim, 2000; Vinci et al., 2001; Patterson et al., 2003). The rest of this review is restricted to separation methods based on density or gravitational forces, including settling tanks and swirl separators.

The driving function in the gravity separation process is the difference in density between the particle and the water. However, a single representative particle density cannot adequately describe aquacultural solids (Wong and Piedrahita, 2000; Patterson et al., 2003). Scott and Allard (1984) and Eikebrokk and Ulgenes (1993) reported that swirl separators, which work based on the density of the particle, removed between 70 and 87% of the particulate matter from a

landbased fish farm recirculation system. Scott and Allard (1984) found that 90% of the particulate wastes produced by trout were larger than 77 micrometers in diameter and that the swirl separator removed 70% of these. With smaller particle sizes, the swirl separator was less efficient, removing only 10% of the waste entering it. Particle load has been found to affect the efficiency of solids removal in sedimentation tanks. Reported SS concentration of untreated aquaculture effluents varied from 1 to 50 mg/L (Bergheim et al., 1993; Kelly et al., 1994; Dumas et al., 1998; Schulz et al., 2003). Bergheim et al. (1998) and Cripps and Bergheim (2000) reported that, depending on the solids load, removal of solids in sedimentation tanks at the same flow rate may be from 58% at about 1 mg SS / min to nearly 90% at 18 mg SS / min, but their effectiveness is limited to particles exceeding 100 µm in diameter.

In the design of sedimentation tanks for aquaculture applications, it has been recommended that water velocity should not exceed 6.7 cm/s (Henderson and Bromage, 1988) to avoid the scouring of settled solids and the risk of turbulence. Raceways can be self-cleaning if water flow velocities along the bottom are greater than 6 cm/s (Burrows and Chenoweth, 1970). Timmons and Youngs (1991) stated that a minimum cleaning velocity can be estimated by the following expression:

$$V_{clean} = \frac{1}{2} d^{4/9}(G - 1)3048 \qquad \text{(Eq. 8.5)}$$

where V_{clean} = cleaning velocity (cm/s)

d = particle diameter (mm)

G = specific gravity of the material

Recommended overflow rates (v_{sc}) are about 1.5 – 3.0 m^3/m^2 h for the removal of ~95% of aquacultural suspended solids (Bergheim et al., 1998). Wong and Piedrahita found that a settling basin designed for an overflow rate of 18 m^3/m^2 h or lower will remove about 80% of the settleable material from flow-through trout farm effluents.

8.2.- EXPERIMENTAL DESIGN

This section describes procedures followed for feeding the fish, collecting the settled solids, and determining the particle settling velocity for solids settled within California halibut rearing vessels operated under farm-like conditions.

8.2.1.- Fish and raceways

California halibut were reared in the raceways described in Appendix A.1. These raceways had a screened off section at the effluent (quiescent zone, QZ) which allowed for solids settling. Settling velocity tests were carried out for mean fish sizes between 0.17 and 108 g wet mass. The fish were raised under farm-like conditions. Tests were conducted between March 2002 and January 2003.

Raceways were cleaned every day at 9:00 h by flushing the solids and scrubbing the tank surfaces. The fish were then fed continuously during 12 h by belt feeders, starting at ~9:30 h. Settleable solids started to accumulate within the QZ at about 17:00 h, almost 7.5 hours after feeding began. Particles used for the settling test were collected at ~ 9:00 h and had settled in the raceways' QZ for a maximum of 14 h.

8.2.2.- Commercial diets

Three commercial diets were used in this study: Nippai "Ambrose 600"® (Nippon Formula Feed Manufactureing Co., Ltd,) for fish from 0.17 to 0.7 g; BioKyowa 1000® (Kyowa Hakko Kogyo Co., Ltd.) for fish from 1.5 to 3.0 g; BioKyowa 2000® for fish from 6 to 40 g; EWOS 2® (EWOS® Canada Limited) for fish from 10 to 20 g; and EWOS 3® for fish from 35 to 110 g. Fish were fed on a percent biomass basis according to the hatchery protocols.

8.2.3.- Settling column

The UFT-type settling column described by Wong and Piedrahita (2000) was used to study the settling properties of solids settled within the raceways in the quiescent zone. Refer to Wong and Piedrahita (2000) for column design details.

8.2.4.- Test protocol

Settling tests were carried out according to the methodology described by Wong and Piedrahita (2000). Particle settling velocity determinations were made in

seawater taken from the recirculating system in which the fish were being cultured. Prior to carrying out the test, the seawater was filtered by method 2540D (APHA, 1995) to remove any suspended particles that might interfere with the analysis, and then poured into the UFT column. Temperature and salinity were recorded at the time of the test as recommended by Wong and Piedrahita (2000).

A portion of the settled solids within the QZ were removed in the morning before the cleaning of the raceways and analyzed immediately for settling velocities. A hollow tube (5 mm diameter) was inserted into the settled solids, the top end was sealed, the tube was removed, and the solids were poured carefully into a pre-weighed graduated glass beaker. The sampling procedure was repeated in different sections of the settled material until about 3 mL of particles were collected. The solids sampled were then weighed to obtain an estimation of the initial wet mass. Before weighing the solids, excess water was carefully removed from the pre-weighed graduated glass beaker. About 1.8 to 3 g of wet mass of the settled material was used for the settling velocity test.

A sample of the solids settled within the QZ for a given raceway was introduced into a short section of acrylic pipe that rested on the bottom of the upper reservoir of the UFT-column. Then the sample was gently stirred, and at time zero the feeding mechanism was quickly slid towards the opening of the UFT column. Samples were withdrawn for 3 seconds at spaced intervals from the cone at the

bottom of the UFT column. Each settling test was performed for up to 120 minutes, with samples withdrawn from the settling column over that time (Fig. 8.2). Each sample withdrawn was analyzed for settleable solids according to Standard Method 2540-D (APHA, 1995). At the end of the experiment all seawater that still remained within the UFT column was also analyzed by Standard Method 2540-D (APHA, 1995).

The sum of the mass of the samples withdrawn within the time of the test was the total amount of mass settled. A percentage of the suspended solids settled within the test frame time was estimated through an accounting of those suspended solids that did not settle. From these results, the cumulative settling velocity curve for each feed and for each fish size was constructed. Settling velocities were adjusted to account for the dropping water levels within the UFT column as explained by Wong and Piedrahita (2000).

8.2.5.- Settling velocity distribution

The expression proposed by Wong and Piedrahita (2000) was used to describe the settling curves obtained in this research (Eq. 8.4). A freeware curve fitting program (WinCurveFit version 1.1.8, June 2002, from Kevin Raner Software http://www.krs.com.au) was used to obtain the suspended solids specific parameters for Eq. 8.4.

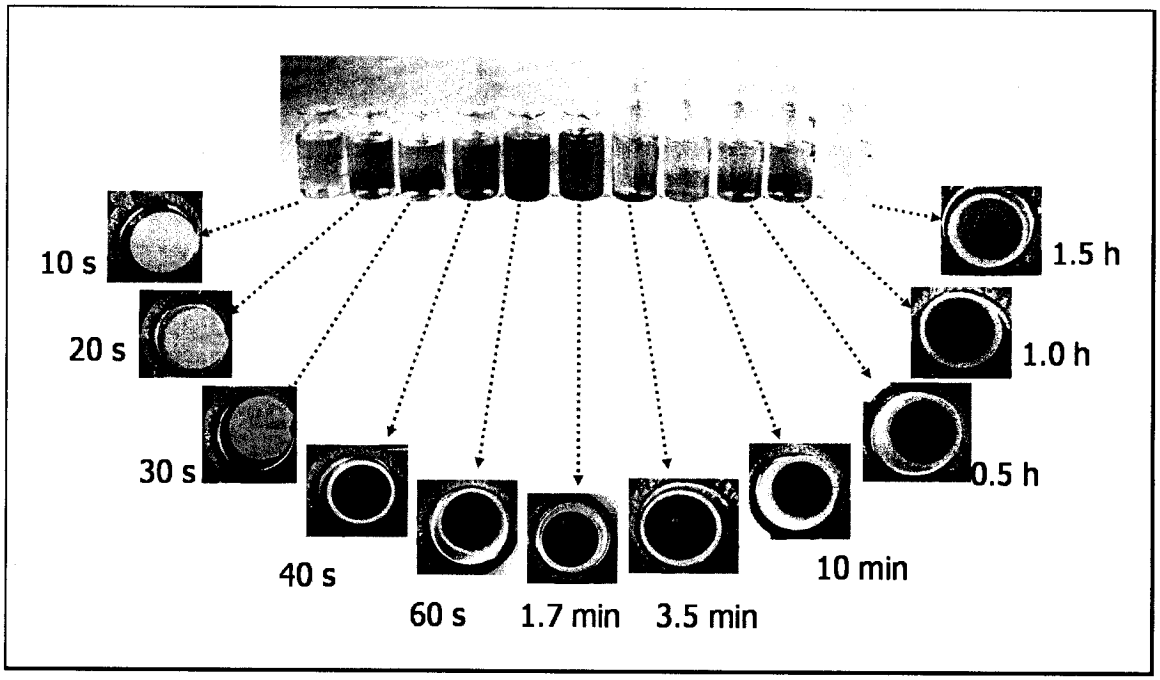

Figure 8.2.- Settled solids samples from the UFT column. Samples for a given time were poured into a glass bottle. Samples were then filtered, dried, and weighed.

8.2.6.- Settling velocity indicators

Settling velocity indicators have been recommended to compare settling velocity distribution curves for storm sewage solids (Michelbach, 1995; Aiguier et al., 1996). Three settling velocity indicators are commonly used and they can be obtained from the settling velocity distribution curves: V_{20}, V_{50}, V_{80}. The settling velocity indicator (Eq. 8.2) is defined as the settling velocity corresponding to a given F_o value (Aiguier et al., 1996; Michelbach and Weib, 1996; Lucas-Aiguier et al., 1998). The settling velocity indicators were calculated by solving for v_s in Eq. 8.4:

$$v_s = \left(\left(\left(\frac{b}{F_o - a}\right) - 1\right)c^d\right)^{1/d} \qquad \text{(Eq. 8.6)}$$

8.2.7.- Overflow rate

OFRs were estimated for the raceways, under normal operation, in this study and then compared to the settling velocity distribution curves to estimate the theoretical overall fraction removal of suspended solids (Eq 8.2). In addition, knowing that the maximum recommended swimming velocity for California halibut is 1.0 bl/s (see Chapter IV), a volumetric flow was estimated and a corresponding OFR was calculated. This OFR was used to estimate the theoretical overall fraction removal of suspended solids under this condition (Eq. 8.2).

8.3.- RESULTS

8.3.1.- Settling solids recovered

The UFT-column allowed the recovery of more than 90% of the solids tested for settling velocity during the time frame used (Table 8.1). An exception, with 84% of recovery, was obtained for the 4 g fish fed with BK1000® (Table 8.1). The lowest overall average recovery was for solids taken from fish fed with EWOS 2®, with 90%.

8.3.2.- Settling velocity distribution.

An example of data for the settling test performed for a raceway stocked with 11 g fish is shown in Table 8.2. The corresponding settling velocity distribution is shown in Fig. 8.3, along with an estimated settling curve using the equation proposed by Wong and Piedrahita (2000).

Table 8.3 shows the equation parameters estimated for all the settling velocity distributions with Eq. 8.4 proposed by Wong and Piedrahita (2000). The correlation coefficients were $0.975 < r^2 < 0.998$.

8.3.3.- Settling velocity indicators

The settling velocity indicators differed for the feeds tested. As an example, the V_{50} indicators observed in this study can be grouped in two categories: from 1.40 to 2.74 cm/s, when fish were fed with either Nippai 600, BK 1000, EWOS 2 or

Table 8.1.- Settling solids recovered. No standard deviation (SD) is shown for tests performed only once.

Fish mean wet mass g	Percentage recovery			Average	SD	Feed type
0.17	97.75	96.07	96.28	96.70	0.92	Nippai 600
0.59	97.87	97.38	97.21	97.49	0.34	Nippai 600
0.68	96.35	95.69	93.84	95.29	1.30	Nippai 600
1.45	97.50	97.22	96.94	97.22	0.28	BK 1000
2.81	98.56	96.91	97.11	97.53	0.90	BK 1000
4.00	86.69	82.02		84.36	3.30	BK 1000
4.00	98.19			98.19		BK 2000
6.00	97.55	97.81	97.34	97.57	0.24	BK 2000
10.00	92.18			92.18		BK 2000
18.57	97.29			97.29		BK 2000
22.15	96.99			96.99		BK 2000
35.38	95.39			95.39		BK 2000
11.00	88.85	92.26	88.38	89.83	2.12	EWOS 2
20.00	95.04	89.54	88.11	90.90	3.66	EWOS 2
35.38	92.44			92.44		EWOS 3
57.82	96.40	97.16	95.96	96.51	0.61	EWOS 3
108.68	97.76	94.33		96.05	2.43	EWOS 3

Table 8.2.- Data from a raceway stocked with 11 g mean mass California halibut. Fish were fed with EWOS 2. Data presented here are averages of three tests.

Sampling event (s)	Mean settling velocity v_s (cm/s)	Mean sample solids (mg/L)	Mean solids fraction	Mean cum. solids fraction	Mean 1 – [cum. solids frac.] F_o	Standard deviation
10	10.1021	0.0030	0.0140	0.0140	0.9860	0.0067
20	5.5067	0.0018	0.0088	0.0227	0.9773	0.0061
30	3.6964	0.0036	0.0171	0.0398	0.9602	0.0097
40	2.7281	0.0305	0.1485	0.1883	0.8117	0.0862
60	1.7879	0.0591	0.2837	0.4720	0.5280	0.1357
100	1.0482	0.0477	0.2273	0.6993	0.3007	0.1058
220	0.4652	0.0279	0.1315	0.8307	0.1693	0.0501
600	0.1635	0.0169	0.0797	0.9104	0.0896	0.0196
1800	0.0521	0.0082	0.0388	0.9493	0.0507	0.0018
3600	0.0248	0.0061	0.0290	0.9783	0.0217	0.0063
5553	0.0152	0.0045	0.0217	1.0000	0.0000	0.0000

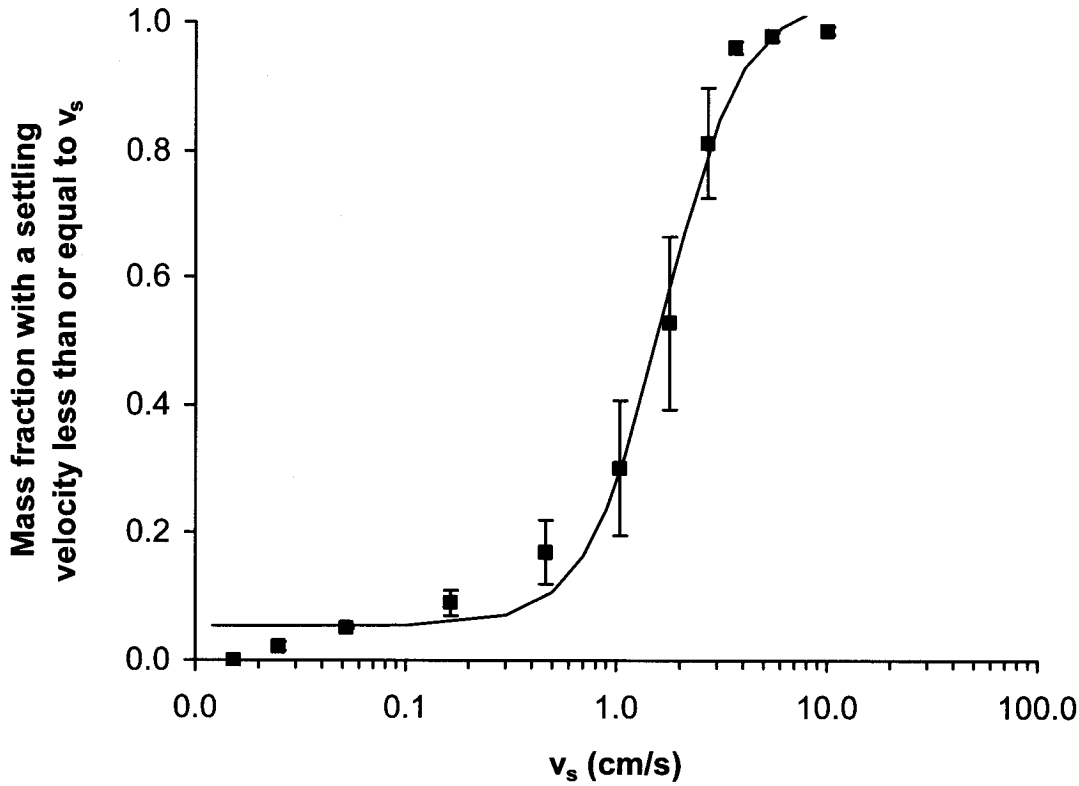

Figure 8.3.- Mass based settling velocity distribution for 11 g fish fed with EWOS 2 based on data shown in Table 8.2. The continuous line shows data estimated from the equation proposed by Wong and Piedrahita (2000). Equation coefficients are listed in Table 8.3. Black square symbols represent mean values of the data observed for this particular test. Vertical lines represent the standard deviation.

Table 8.3.- Parameters for Eq. 8.4 used to describe the settling velocity distribution curves in this study.

Fish mean wet mass	Equation coefficients					Feed type
g	*a*	*b*	*c*	*d*	r^2	
0.17	1.00688	-0.97091	1.45573	2.50978	0.997	Nippai 600
0.59	1.01225	-0.98341	1.49423	2.78387	0.996	Nippai 600
0.68	1.01600	-0.9646	2.38082	2.30929	0.993	Nippai 600
1.45	1.00601	-0.96256	2.33518	3.27708	0.995	BK 1000
2.81	1.04337	-1.00534	2.68292	2.59588	0.995	BK 1000
6.00	1.00267	-0.96035	4.35465	5.86211	0.992	BK 2000
10.00	1.02466	-0.97352	4.03496	4.1895	0.987	BK 2000
22.15	0.99615	-0.93372	4.7553	5.63928	0.987	BK 2000
35.38	0.99349	-0.90498	4.40117	5.68655	0.975	BK 2000
11.00	1.03584	-0.98203	1.68396	2.35997	0.990	EWOS 2
20.00	1.02615	-0.97372	1.92015	2.82051	0.991	EWOS 2
35.38	1.18883	-1.15879	2.83999	1.50786	0.984	EWOS 3
57.82	1.05024	-1.00588	2.91912	2.91801	0.986	EWOS 3
108.68	1.09063	-1.07628	1.19644	1.20886	0.998	EWOS 3

EWOS 3; and from 3.89 to 5.05 cm/s, when fish were fed with BK 2000. Suspended solids produced from fish fed with BK 2000 presented the highest settling velocities for all solids settling indicators (Table 8.4).

Settling velocity indicators change with the type of feed but not with fish mass (Table 8.4). Fish of 4 g fed with BK 2000 produced suspended solids that settled more quickly than when they were fed with BK 1000. Fish 10 to 11 g fed with BK 2000 produced suspended solids that settled more quickly than when being fed with EWOS 2. Fish between 18 and 22 g fed with BK 2000 produced suspended solids that settled more quickly that when they were fed with EWOS 2. And 35 g fish fed with BK 2000 produced suspended solids that settled more quickly than when they were fed with EWOS 3.

8.3.4.- Overflow rate and solids settling

Raceways under normal operation during this research had OFRs under 0.04 cm/s or 1.36 m^3/m^2 h. At these low OFRs between 98 and 100% of the suspended solids were estimated to settle within the rearing tank (Table 8.5). From Chapter IV, it was learned that California halibut can grow well up to a relative swimming velocity of 1 bl/s. Estimated OFRs for raceways having a relative swimming velocity of 1 bl/s were between 0.16 and 0.31 cm/s (5.78 and 11.10 m^3/m^2 h). For these estimated OFRs between 90 and 98% of the suspended solids were estimated to settle within the rearing unit (Table 8.5). OFRs for a relative swimming velocity of 1 bl/s are between 6.6 and 13.5 times larger than OFRs under normal operation.

Table 8.4.- Settling velocity indicators.

Fish mean wet mass	Settling velocity indicators (cm/s)						Feed Type
g	V_{20}	SD	V_{50}	SD	V_{80}	SD	
0.17	0.77	0.16	1.41	0.23	2.45	0.47	Nippai 600
0.59	0.86	0.09	1.45	0.11	2.38	0.13	Nippai 600
0.68	1.14	0.03	2.24	0.11	4.08	0.35	Nippai 600
1.45	1.42	0.16	2.27	0.18	3.48	0.18	BK 1000
2.81	1.42	0.38	2.52	0.40	4.17	0.30	BK 1000
4.00	0.98	0.10	1.81	0.05	3.02	0.09	BK 1000
4.00	2.78		5.05		7.66		BK 2000
6.00	3.30	0.95	4.29	0.42	5.46	1.47	BK 2000
10.00	2.69		3.89		5.38		BK 2000
18.57	3.32		4.32		5.51		BK 2000
22.15	3.49		4.65		6.02		BK 2000
35.38	3.12		4.27		5.54		BK 2000
11.00	0.81	0.34	1.56	0.39	2.75	0.34	EWOS 2
20.00	1.05	0.09	1.81	0.03	2.94	0.18	EWOS 2
35.38	0.89		2.21		4.47		EWOS 3
57.82	1.64	0.07	2.74	0.26	4.27	0.57	EWOS 3
108.68	0.33		1.02		2.73		EWOS 3

SD: standard deviation. No SD is shown in for test performed only once.

Table 8.5.- Theoretical overall fraction removal of suspended particles within the California halibut culture raceways. Normal raceway operation refers to the OFR conditions at which the vessel was working at the time of this research. Hypothetical raceway operation refers to an OFR estimated if the raceways were working at the suggested maximum swimming velocity of 1 bl/s (Chapter IV). Theoretical overall fraction removals of suspended particles were calculated with Eq. 8.2.

	g/fish				
	0.59	2.8	10	11	35
			Feed offered		
	NIPPAI	BK	BK	EWOS	EWOS
	600	1000	2000	2	3
Normal raceway operation					
Flow (L/min)	7.5	9.0	10.5	10.5	15.0
Velocity (cm/s)	0.5	0.5	1.3	1.3	1.8
OFR (cm/s)	0.02	0.02	0.03	0.03	0.04
OFR (m^3/m^2 h)	0.68	0.81	0.95	0.95	1.36
η (%)	99	100	100	98	98
Hypothetical raceway operation at 1 bl/s					
Flow (L/min)	63.8	106.6	81.1	83.7	122.6
Velocity (cm/s)	3.9	6.5	9.8	10.1	14.9
OFR (cm/s)	0.16	0.27	0.20	0.21	0.31
OFR (m^3/m^2 h)	5.78	9.65	7.34	7.58	11.10
η (%)	96	94	98	91	90

8.4 DISCUSSION

The settling of solids within a culture unit is not desirable, since the health of the fish can be threatened by the decomposition of this waste product. This can be a result of the direct effect of the solids on the fish, or indirectly by allowing a suitable substrate for the growth of pathogenic bacteria or an increase in the consumption of dissolved oxygen and ammonia-N generation as solids decay (Timmons et al., 2001). For an aquacultural recycle system, an increase in oxygen consumption and ammonia-N due to solids mineralization may affect the efficiency of unit operations within the culture system (Losordo, 1991; Chen et al., 1992).

8.4.1.- Settling solids recovered

An essential step in the design of a self-cleaning tank and an adequate solids removal system is the characterization of the particulate material to be removed (Timmons and Youngs, 1991). This study gathered information on settling properties of particles produced by California halibut juveniles. Settling velocity experiments were performed in an UFT (Wong and Piedrahita, 2001) column with solids that were already settled in the quiescent zone within the raceways. During the test a minimum of 90% of the solids were recovered within the time frame studied.

8.4.2.- Settling velocity distribution

As suggested by Wong and Piedrahita (2001), Eq. 8.4 was used to describe the settling velocity distributions. For this study Eq. 8.4 was found to fit the data with a correlation coefficient between 0.975 and 0.998. Parameters *a* and *b* are quite similar for all the settling curves, but parameters *c* and*d* d vary with the type of feed (Table 8.3). Fig. 8.4 shows that suspended solids having settling curves with larger parameters *c* and *d* should be removed more effectively by settling basins than would those with smaller parameters. A smaller parameter *c* moves the curve to the left, indicating slowly settling particles. The steepness of the curve is related to the magnitude of parameter *d* and a larger *d* describes a curve with a steeper slope. Consequently, for settling velocity distributions having a high *d* parameter, a very small change in v_s (or OFR), within a certain OFR range, should result in very large changes in solids removal by settling.

The settling properties of the particles that settled within the raceways were found to be likely linked to the type of feed offered and not to the size of the fish under culture. In addition, feces stability in the water was observed to be related with the type of feed offered. The Japanese diets (Nippai™ and BioKyowa™) yielded fecal particles that were more stable in water than did the Canadian diet (EWOS™). Hence a greater proportion of fine solids can be expected to be suspended in the water when fish are fed with EWOS™ than when fed with BioKyowa™.

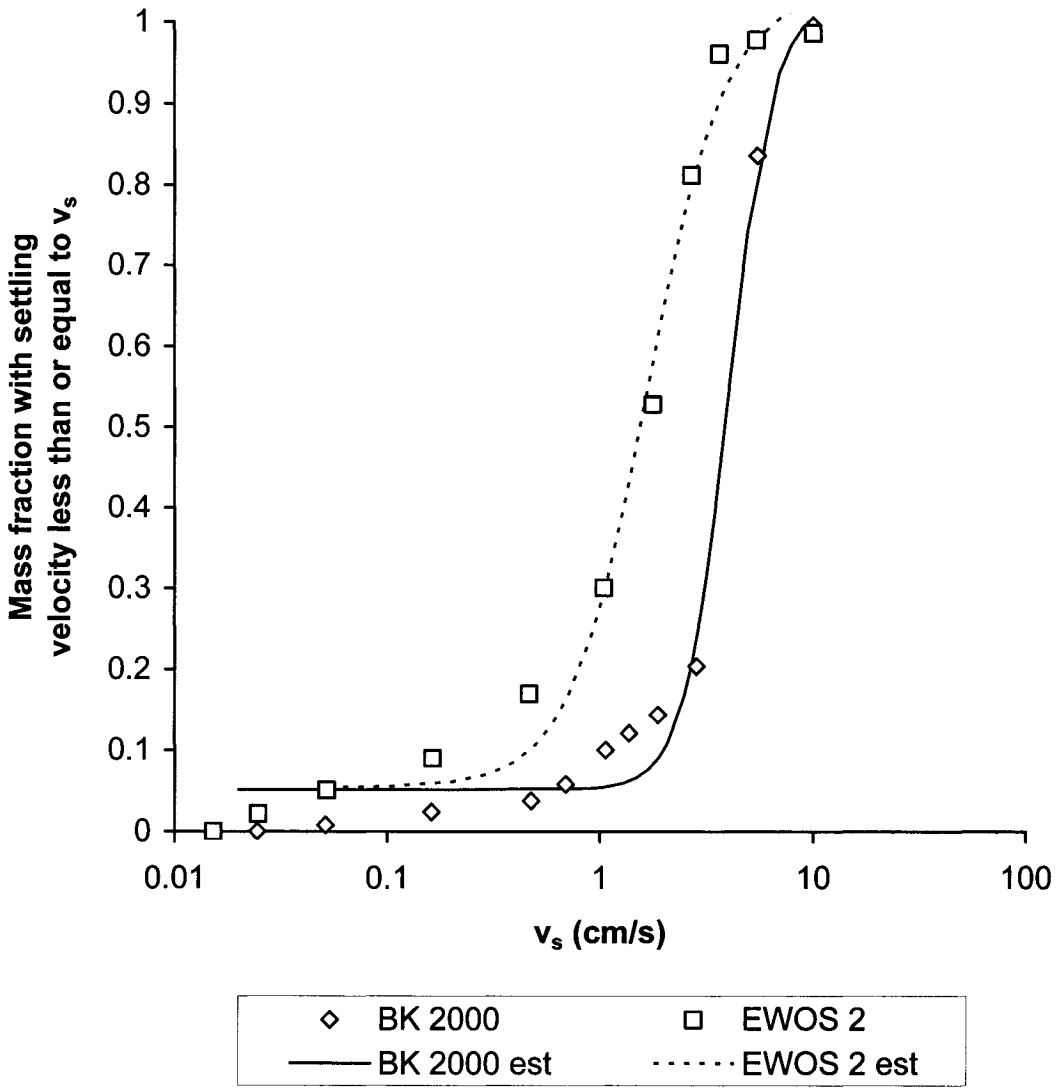

Figure 8.4.- Settling velocity distributions for two types of solids produced for 10.00 g and 11.00 g fish fed with BK 2000 and EWOS 2, respectively. Symbols represent data points. Lines show curves estimated using Eq. 8.4 (Wong and Piedrahita, 2000). Equation coefficients are listed in Table 8.3.

8.4.3.- Settling velocity indicators

Typically, settling velocity distribution curves are produced as cumulative graphs which show the proportion of material by mass with settling velocities less than a given settling velocity. From settling velocity distribution curves several settling velocity indicators can be obtained, such as V_{20}, V_{50}, and V_{80}.

Aquaculture solids have varying settling velocity (v_s) values depending on their size and specific gravity. Trout fecal casts are large and heavy, and will have settling velocities ranging from 2.0 to 5. 0 cm/s, but fine, lighter particles settle at much slower rates ranging from 0.05 to 0.09 cm/s (Fornshell, 2001). In the present study, V_{50} values ranged between 1.02 and 5.05 cm/s (Table 8.4). Particles produced from California halibut fed with BK 2000 had the highest V_{50} values, between 3.89 and 5.05 cm/s, among all the feeds tested (Table 8.4).

The settling velocity distributions obtained in this study are compared to those published by Wong and Piedrahita (2000) (Fig. 8.5). The results for EWOS 3 are very similar to those of Wong and Piedrahita (2000) and indicate solids that should be more difficult to remove by settling than those produced by fish fed with EWOS 2 or BK feeds.

8.4.4.- Overflow rate and solids settling

When designing a sedimentation tank, Eq. 8.2 should be used to get a prediction of the overall fraction of solids removal. On this study, Eq. 8.4 proposed by Wong

and Piedrahita (2000) was found to fit the settling solids distribution curves for the suspended solids analyzed. The Eq. 8.4 was used to solve numerically Eq. 8.2, and obtain estimates of the theoretical overall removal of SS produced by any of the feeds offered to California halibut for a given OFR (Table 8.6).

During this study, it was observed that SS tend to settle along the raceways when fish were stocked at about 100% PCA. However, when the raceways were stocked over 150% PCA, the settled solids were resuspended by the fish activity and carried towards the QZ. In this study the OFRs for the normal raceway operation were between 0.02 and 0.04 cm/s. At these OFRs about 100% of SS, recovered in the UFT column tests, will be expected to settle out (Table 8.5).

During the experiment, raceways were not operated at the maximum water velocity allowed by fish size (Chapter IV). A higher water velocity will result in higher OFRs in a rearing tank. At larger OFRs fewer solids will settle within the rearing unit. At the maximum water velocity recommended for California halibut (1 bl/s, Chapter IV) the OFRs will be between 0.16 and 0.31 cm/s (Table 8.5). At these OFRs between 90 and 98% of the solids mass fraction will settle within the raceways or in the quiescent zone, depending on fish stocking density (Table 8.5).

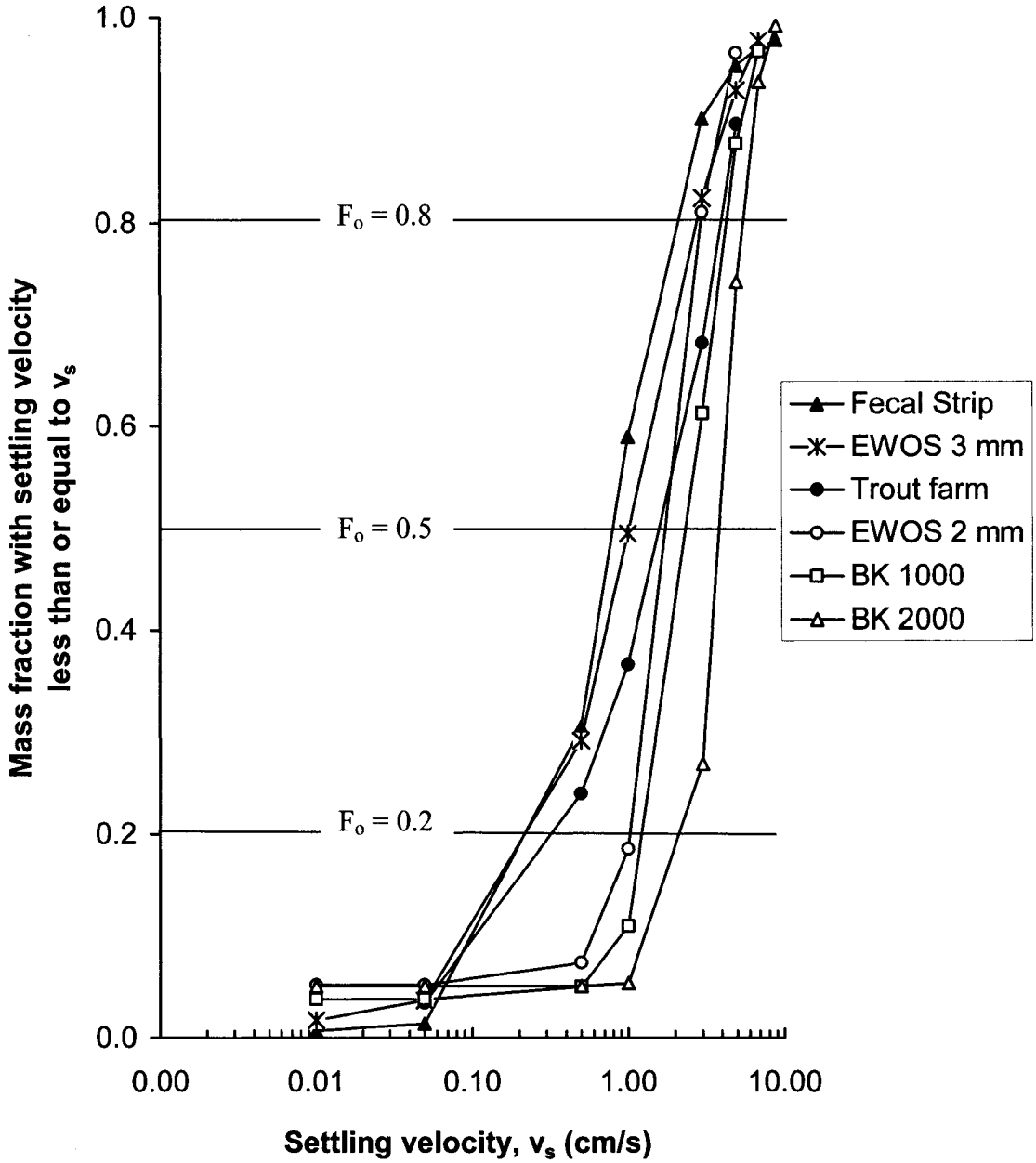

Figure 8.5- Mass based settling velocity distribution curves. Values shown for EWOS 3 mm are for 108.7 g fish, EWOS 2 mm for 20 g fish, BK 1000 for 2.81 g fish, and BK 2000 for 10 g fish. Fecal strip and trout farm data taken from Wong and Piedrahita (2000).

Table 8.6.- Effect of OFR on the theoretical fraction of solids removed by settling. Suspended particles were produced by California halibut fed with different feed sizes according to fish size.

	Fish mean wet mass (g)				
	0.59	2.8	10	11	35
	Feed offered				
	NIPPAI 600	BK 1000	BK 2000	EWOS 2	EWOS 3
OFR	Theoretical overall fraction removed (Eq. 8.2)				
cm/s	%	%	%	%	%
7	19	32	42	20	29
6	22	38	49	24	34
5	26	44	57	28	41
4	32	54	67	34	50
3	42	66	84	45	61
2	58	78	90	60	70
1	80	89	94	77	80
0.5	91	93	97	85	88

8.5.- CONCLUSIONS

The most relevant conclusions from the present chapter are:

a) In view of the large number of parameters affecting particle settling velocity (ie., particle density, particle shape) and the variation of physical characteristics (ie., water temperature, salinity) among aquacultural wastewaters, it is advisable to conduct *in situ* analyses for a direct measurement of the property to be used for removal. Direct measurement of particle settling velocity does away with the needs for many assumptions related to particle and water physical properties.

b) The technique described by Wong and Piedrahita (2000) for measuring settling velocity is convenient and rapid. The UFT settling type column built by Wong and Piedrahita (2000), and used in the present research, recovered about 90% of the solids settled within the California halibut culture raceways for the time frame tested.

c) Settling velocities of settleable solids were analyzed for California halibut juveniles fed with various types of feed. The measurements indicate that solids produced by feeding fish with BioKyowa^{TM} pellets had the fastest settling velocity among the diets tested.

d) It was observed that stocking the raceways with California halibut with at least a 150% PCA helps to resuspend the settled solids and maintain a cleaner culture tank.

e) The experimental flow rates used in the raceways led to OFRs between 0.02 and 0.04 cm/s. At these low OFRs, the solids analyzed would be expected to settle within the culture tanks if stocking density is less than 100% PCA, or in the quiescent zone if the stocking densities are over 150% PCA.

f) Settling velocities were related to the type of feed offered rather than to the size of the fish. This is important, as water velocities can be adjusted up to a limit of 1 bl/s without affecting fish growth, and consequently velocity can be increased as the fish grow. An increase in OFR will reduce the settling of solids within the raceways and make them self cleaning.

g) The equation proposed by Wong and Piedrahita (2000) for describing the settling velocity distributions explained ~99% of the data obtained in this research. This equation facilitates the analysis of the removal efficiency expression used to design settling basins.

h) The settling velocity curves obtained in this research contain valuable information that can be used to select appropriate devices for the removal of solids. Settling basins and swirl separators appear to be the best option for the immediate removal of particles coming out from the culture vessels, due to their relatively high settling velocities.

8.6.- FURTHER RESEARCH

Further studies are recommended in the following areas:

- Studies should be performed on the leaching of nutrients from California halibut feces when the fish are fed with different types of feed.

- Settling distribution curves based on BOD, C, N, and P should be developed.

- Particle size distribution should be determined so that the effectiveness of solids removal by screening or filtering can be evaluated.

CHAPTER IX

NITRIFICATION PERFORMANCE OF A SUBMERGED MOVING BED BIOFILTER

9.1.- INTRODUCTION

Numerous scientific and commercial ventures have demonstrated the technical feasibility of raising a wide variety of aquacultural species under a water recirculating regime (Broussard and Simco, 1976; Bovendeur et al., 1987; Eikebrokk and Piedrahita, 1997). In the design of recirculating aquaculture systems, the major unit operations (or processes) to consider are aeration/oxygenation, ammonia removal, and solids removal (Lawson, 1995; Twarowska et al., 1997; Losordo et al., 2000; Timmons et al., 2001). Nitrification processes have been proved to substantially reduced water use in aquaculture systems (Timmons et al., 2001). Typically, fixed film processes are for ammonia removal by nitrification (Brune and Gunther, 1981; Kaiser and Wheaton, 1983; Rogers and Klemetson, 1985; Losordo et al., 2000; Timmons et al., 2001). Many materials are used as biomedia to provide an adequate substrate for growing nitrifier bacteria. Among the materials used are sand, gravel, and plastic (Speece, 1973; Muir, 1982; Manthe, 1991; Kikuchi et al., 1994; Wheaton et al., 1994; Sandu et al., 2002).

Originally, biofilters were utilized in municipal wastewater systems, where ammonia concentrations are usually high (typically greater than 17 mg TAN/L) (Metcalf & Eddy, Inc., 1991). In recirculating aquaculture systems, ammonia concentrations are much lower (typically under 1 mg TAN/L) (Guger and Boller, 1986; Twarowska et al., 1997), resulting in TAN removal rates below the removal rates of similar biofilters in municipal wastewater systems (Brune and Gunther, 1981). To design an effective recirculating aquaculture system it is important to examine the nitrifying performance of biological filters (Timmons et al., 2001).

In aquaculture systems, nitrification biofilters must remove TAN at a sufficient rate to maintain water quality at a level adequate to prevent TAN toxic exposure to the fish (Alderson, 1979; Wajsbrot et al., 1993; Person-Le Ruyet et al., 1997). Waters in actual recirculating aquaculture systems usually contains high BOD (biochemical oxygen demand) concentrations that provide substrate for heterotrophic bacteria which compete with nitrifiers for growing space, oxygen and other nutrients that can have an impact on biofilter performance (Heinsbroek and Kamstra, 1990; Eikebrokk and Piedrahita, 1997). However, most nitrification studies are carried out under controlled conditions in the laboratory and TAN removal data gathered in these studies could be thus much higher than in commercial systems (Lu and Piedrahita, 1993; Zhu and Chen, 1999). The present research will characterize the nitrification performance of a marine submerged moving bed biofilter under farm-like conditions in a recirculating aquacultural system for California halibut rearing. The results of this study will be

useful in the design and application of submerged moving bed biofilters for marine recirculating system operations.

9.1.1.- Literature review

The TAN oxidation rate in a biofilter is affected by the characteristics of the filter media as well as by the operating conditions for the biofilter, such us pH, temperature, hydraulic loading, and dissolved oxygen (Weatherley, 1984; Nijhof, 1995; Timmons et al., 2001). This literature review will focus on the most important factors affecting the performance of a biofilter: the ranges of TAN removal for the most used biofilters in aquaculture, the description of a submerged moving bed biofilter, and methodologies used to measure biofilter nitrification rates within a farm-like system.

9.1.1.1.- Chemical factors affecting biofilter performance

Nitrification is an acid-forming process; hence pH will decline if the water is poorly buffered (Timmons et al., 2001). A significant decline in nitrification kinetics occurs at pH values just below neutral (Malone and DeLosReyes, 1997). Therefore, proper pH management is essential for obtaining the optimum nitrification performance in water recirculating systems (Loyless and Malone, 1997).

Nitrification consumes alkalinity at a rate of 0.14 meq/mg TAN oxidized to nitrate (Timmons et al., 2001). A significant decline in nitrification kinetics occurs with

alkalinities below 1.3 meq/L (Malone and DeLosReyes, 1997). According to Kikuchi et al. (1994), ammonia oxidation was inhibited when pH and alkalinity reached 6.0 and 0.5 meq/L, respectively. Loyless and Malone (1997) recommended maintaining pH in the range of 7.5 to 8.0 and an alkalinity of ~3-4 meq/L in high-density grow out recirculating systems. Also it has been reported that nitrification rate is independent of pH in the range of 6.5 to 8.0 if alkalinity is over ~1.6 meq/L (Speece, 1973; Malone and DeLosReyes, 1997).

Nitrification rates decrease when insufficient oxygen is available to the nitrifying bacteria (Nagel and Haworth, 1969; Kaiser and Wheaton, 1983). Speece (1973) stated that the nitrification rate was independent of dissolved oxygen concentration as long as the stoichiometric requirement was met; about 4.57 mg O_2/mg TAN are required (Timmons et al., 2001).

9.1.1.2.- Physical factors affecting biofilter performance

Biofilter efficiency also is affected by temperature (Timmons et al., 2001; Zhu and Chen, 2002). Wortman and Wheaton (1991) determined that the nitrification in a biodrum had a linear relationship both for TAN removal rate and for nitrate production rate at temperatures between 7 and 35 °C. Zhu and Chen (2002) also stated that temperature between 8 and 27 °C had a significant effect on nitrification rate. The limited information available on the effect of temperature on nitrification rate suggests that nitrifying bacteria are able to adapt to a wide range of environmental temperatures to perform nitrification.

Nitrification rates in a biofilter have been shown to depend on TAN and on dissolved oxygen concentrations and diffusion rates (Kaiser and Wheaton, 1983; Timmons et al., 2001). The concentration ranges at which TAN and oxygen become rate limiting depend on biofilm properties and diffusion rates (Kugaprasatham et al., 1991; .Zhu and Chen, 1999; 2003). Relatively high flow rates cause greater shearing forces, which reduce the biofilm thickness and lessen the distance the soluble material has to diffuse. Zhu and Chen (2001) reported that the TAN removal rate can be improved by increasing the Reynolds number of the flow over the biofilm surface; the TAN removal rate at a Reynolds number of 66710 was five times that at a Reynolds number of 1668. Sandu et al. (2002) stated that biofilm thickness increased with TAN loading (biofilm thickness was 44 μm at 180 mg TAN/L and 90 μm at 360 mg TAN/L, both at at 12 L/min), but decreased with increased hydraulic loading rates (biofilm thickness was 76 μm at 6 L/min and 44 μm at 12 L/min).

Hydraulic loading rates and hydraulic residence time can affect a biofilter performance. Hydraulic loading rate is a measure of the volume of water flowing through a biofilter per unit of cross sectional area of filter bed per unit of time (m^3/m^2 d). Hydraulic residence time is the average time a particle or volume element resides in a reactor. Brune and Gunther (1981) showed that high nitrification rates in a biofilter were possible at short hydraulic residence times and low ammonia concentrations if the bacterial numbers were high. Kaiser and Wheaton (1983) observed that for low ammonia concentrations shorter hydraulic

residence times (higher flow rates) produced higher ammonia mass removal rates. Lu and Piedrahita (1993) reported that increasing the hydraulic loading rate (188 up to 3765 m^3/m^2 d) for a trickling filter resulted in an improvement in biofilm formation and in the nitrification rate. Sae et al. (2001) reported that the ammonia removal rate was highest when the hydraulic residence time was 1 h (for a range between 6.12 and 0.7 h). Kaiser and Wheaton (1983) argued that the nitrifiers growth rate and the nitrification rate were not a function of the concentration of the limiting substrate, but were a function of the mass loading of the limiting substrate.

Filter media is the most sensitive variable when designing biofilters (Lekang and Kleppe, 2000). There are hundreds of media types that may be used for nitrification filters, ranging from sand and rocks to plastic media (Speece, 1973; Muir, 1982; Kikuchi et al., 1994; Wheaton et al., 1994; Westerman et al., 1996; Kamstra et al., 1998; Ridha y Cruz, 2001; Sandu et al., 2002). Almost any solid material that is non-toxic to the nitrifiers and the crop can be used as media. The selection of a media depends on cost, weight, surface area per volume, void ratio, availability, and system loading (Timmons et al., 2001).

9.1.1.3.- Biofilters TAN removal rates

Studies of biofilters in aquaculture have reported comparisons between various filter types (Table 9.1). The TAN removal efficiency for a rotating biological contactor (RBC) was found to be between 74 and 82%; for a biodrum, ~80%; for

a tricking filter, between 23 and 52%, and for a fluidized sand bed filter, between 8 and 32% (Miller and Libey, 1985; Rogers and Klemetson, 1985). Van Rijn and Rivera (1990) showed that the maximum removal rate of TAN in a trickling filter with PVC media was 0.43 g TAN/m^2/day. Nijhof and Bovendeur (1990) reported that the maximum nitrification capacity of a filter with a plastic filter medium was 0.28 g TAN/m^2/day. Westerman et al. (1993) reported 0.25 g TAN/m^2/d for an RBC and 0.1 to 0.15 g TAN/m^2/d for up-flow sand and bead filters. Kikuchi et al. (1994), studying five kinds of plastic filter media, reported a maximum ammonia oxidation rate of 0.55 g TAN/m^2/day for a biofilter with net filter medium. Twarowska et al. (1997) stated that a high-rate linear-path trickling biological filter had an average removal rate per unit of filter surface area of 0.33 g TAN/m^2/day. Eikebrokk and Piedrahita (1997) reported between 0.33 and 0.66 g TAN/m^2/day for an up-flow submerged biofilter. For a description of the biofilter configurations mentioned here, the reader should review Timmons et al. (2001).

9.1.1.4.- Submerged moving bed biofilm reactor

The submerged moving bed biofilm bioreactor is a variation of the traditional submerged biofilters. Traditional submerged biofilters consist of a bed of fixed media, upon which biomass grows, through which the water passes (Timmons et al., 2001). In the moving bed biofilm reactor (MBBR) the biomass grows on small carrier elements that move along with the water in the reactor (Hem et al., 1994). Air is usually introduced at the bottom of the reactor, mixing the reactor contents and keeping carriers, which have a density close to that of water, in suspension

Table 9.1.- Biofilter physical properties and nitrification rates reported for aquaculture systems.

Biofilter type	SSA m^2/m^3	V m^3	HLR L/m^2 min	TAN conv g TAN/m^2 d	NO_2-N conv g NO_2-N/m^2 d	Authors
Trickling biofilter	200	7.7	174	0.43		Losordo et al., 2000
Rotating biological contactor	370	1.27	160	0.11 to 0.44	0.23 to 0.83	Westerman et al., 1993
Up flow sand biofilter	3000	0.13	690	0.04 to 0.18	0.16 to 0.58	
Bead biofilter	1200	0.28	230	0.05 to 0.21	0.00 to 0.16	
Trickling biofilter				0.43		Van Rijn and Rivera, 1990
Trickling biofilter	416	2	167			Twarowska et al., 1997
Trickling biofilter	416	2	318	0.33	0.107	
Rotating biological contactor				0.28		Malone et al., 1993
Bead biofilter				0.29		
Fluidized bed filter	1600	0.01	96	0.23		Sandu et al., 2002
	1600	0.01	66	0.27		
	1600	0.01	48	0.27		
Upflow submerged biofilter	230	0.53		0.66		Eikebrokk and Piedrahita, 1997
	230	0.65		0.31		

SSA = specific surface area; V = filter volume; HLR = hydraulic loading rate.

Empty columns means no data given by the consulted source.

and continuous movement. The MBBR requires heavy aeration relative to traditional submerged biofilters. The heavy aeration is needed to maintain the bed in motion and to minimize dissolved oxygen problems and solids accumulation (Rusten et al., 1998). A screen is provided at the outfall end of the reactor to keep media from clogging the effluent or passing out of the reactor. A MBBR is a self-cleaning, low maintenance biofilter requiring no backwashing, with low head loss that is easy to build and maintain (Rusten et al., 1995; Asiedu, 2001).

The Norwegian University of Science and Technology (NTNU) and Kaldnes Miljoteknologi A/S (KMT) of Norway developed a plastic media made of polyethylene which is been widely used for MBBR technology (www.kmt.no; www.kaldnes.com). The medium is shaped in a form of a wheel and has a width of 7 mm and diameter of 10 mm. The Kaldnes^{TM} media is provided with fins on the outside to prevent biofilm loss and promote growth of biofilm. The effective area of the Kalnes^{TM} medium is reported to be 70% of the total surface area due to lower attachment of biofilm on the outer perimeter of the media. The effective specific area of the medium is 500 m^2/m^3. The percent of reactor volume comprised of media is limited to 70%, with 67% being typical (Odegaard et al., 1994; Asiedu, 2001). The average nitrification rate for Kaldnes^{TM} in municipal waste treatment plants amounted to 107 g TAN/m^3d (at 5 g O_2/m^3, 10 °C and 60% filling ratio) (Maurer et al., 1999).

Zhu and Chen (1999) determined at a laboratory scale that the maximum nitrification rate by a MBBR using Kaldnes^{TM} media at 27.2 °C can be determined by the following equation:

$$R = 1859 * \frac{S - 0.07}{S + 1.93} \qquad \text{(Eq. 9.1)}$$

where, R = TAN removal rate (mg/m^2 d)

S = TAN concentration (mg/L)

Factors reported to affect performance are flow and mixing conditions in the reactor (Rusten et al., 1998). Turbulence is needed for efficient system performance and to slough off excess biomass and maintain an adequate biofilm thickness (~100 μm). Excessive turbulence detaches biomass from the carrier and therefore is not recommended (Asiedu, 2001).

9.1.1.5.- Measurement of nitrification rates

The TAN conversion rates are used for evaluation and comparison of biofilter performance. Mass balances applied either to a biofilter or to a recirculating system have been used to determine TAN removal rates for biofilters under farm-like conditions (Westerman et al., 1996; Eikebrook and Piedrahita, 1997; Twarowska et al., 1997; Losordo et al., 2000; Suzuki et al., 2000)

Mass balances approaches to a biofilter have been used to determine biofilter nitrification. Losordo et al (2000) determined that the nitrification rates of a trickling biofilter under farm-like conditions was 0.43 g TAN/ m^2 d. These nitrification rates were estimated by multiplying the average flow rate (L/d) to the filter by the difference between the biofilter inflow and outflow TAN concentration (g/L), and then dividing by the total area (m^2) of the biological filter media. Twarowska et al. (1997) evaluated the TAN removal rate per day of a trickling biofilter by sampling the influent and effluent every 4 h during a 24 h study. The trickling biofilter was operated at two flow rates: 246 and 469 L/min. TAN removal efficiencies (based on in and out samples) was higher at the lower flow rate (80.5±8.0%) than at the higher flow rate (64.8±19.7%). Sanda et al. (2002) also described for a fluidized bed filter that the percent of TAN removed per pass decreased significantly as flow rate increased.

Mass balances approaches to a whole recirculating system have been also used to determine biofilter nitrification. Eikebrook and Piedrahita (1997) estimated the nitrification rates of an up-flow fixed submerged biofilter within a farm-like salmon smolt culture system. Eikebrook and Piedrahita (1997) used mass balance calculations on TAN for the whole recirculating system to estimate the nitrification rate, which was between 0.31 and 0.66 g TAN/m^2 d. Eikebrokk and Piedrahita (1997) also found that the rate of nitrification in the up-flow fixed submerged biofilter increased with the TAN load. Westerman et al. (1993) also determined a biofilter performance within an intensive recirculating fish production facility by

taking water samples every 4 h during the day. The TAN concentration reported by Westerman et al. (1993) varied from 1.5 to 3 mg/L while NO_2-N was 1.0 to 1.2 mg/L. The highest TAN and NO_2-N removal rates were when the concentrations were highest. Westerman et al. (1993) recommended that samples be taken several times a day to get a better representation of a biofilters performance.

9.2.- EXPERIMENTAL METHODS

The experimental seawater recirculating system is presented in details in Appendix A.1and in Fig. 9.1. The recirculating system has an operational volume of 2860 L, however during the biofilter performance studies an additional experiment was set up (Chapter V), which increased the system volume up to 3000 L. Aeration was provided in the biofilter by airstones to keep the effluent oxygen concentration at ~6.5 mg/L. System water temperature was 21 ± 1 °C, pH 7.5 ± 0.3, and salinity 31 ± 1 g/L.

The seawater used as make-up water was trucked from the Bodega Marine Laboratory and poured into a holding tank (Appendix A.1). In the holding tank the seawater was chlorinated/de-chlorinated and continuously filtered (10 and 5 µm). A total of 30 L of seawater were exchanged per day (15 L at 9:00 and 15 L at 17:00 with seawater from the holding tank.

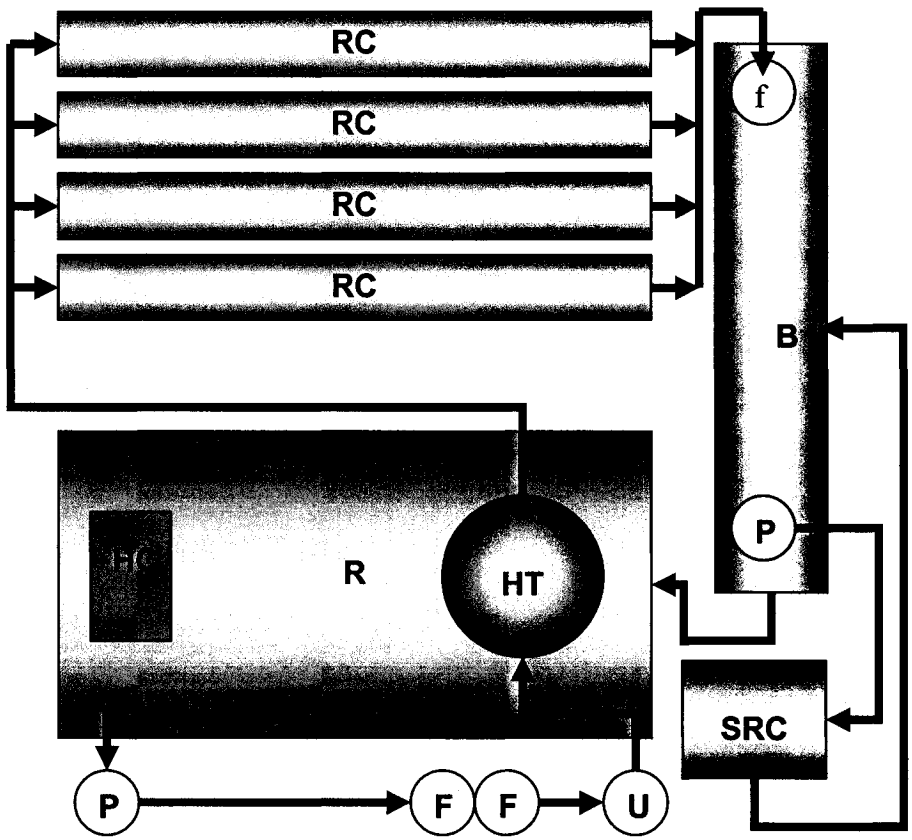

Figure 9.1.- Diagram of the experimental seawater recirculating system used for nitrification tests. Total water volume was 3000 L. P = pump; F = cartridge filter 20 µm; U = ultraviolet light; HT = head tank (100 L); R = reservoir (2160 L); RC = raceway (135 L for all); f = bag filter 50 µm; B = Biofilter tank (466 L); SRC = attached experiment (139 L).

9.2.1.- Diets

Diets used to feed the fish were those manufactured by EWOS™ Canada Limited. Table 9.2 displays the nutritional characteristics of the EWOS™ feed used.

9.2.2.- Culture protocol

Fish biomass was recorded every other week to adjust the amount of feed to a ratio of 1.2 % body mass. The fish were fed commercial dry pelleted feeds (EWOS™ 1 and 2 mm) by 12-h automatic belt feeders during the light time (09:00 to 22:00 h) 7 d/wk. Water flow rates within the raceways were adjusted to have ~5 mg/L of dissolved oxygen at the effluent point. Raceways were cleaned daily in the morning before feeding. The cleaning process consisted of manually scraping the tank bottom, from the influent to the effluent section, to remove biofouling and dead fish.

9.2.3.- Filter media and biofilter tank

A mixture of three supporting media was used as substrate to grow nitrifier bacteria in the biofilter tank: WMT Kaldnes™ 10 mm media (Table 9.3); Rauschert™ Bioflow™ 9 mm media and Biolox™ 10 mm media (Table 9.4). The volume of the biomedia was 140 L, distributed as 25 L for Bioflow™ 9 mm media, 25 L for Biolox™ 10 mm media, and 90 L for WMT Kaldnes™ 10 mm media. The total media surface area was 82.3 m^2.

Table 9.2.- Nutritional characteristics of feed used (EWOS Canada).

Type	Contents
Crude protein	43 %
Crude fat	14 %
Crude fiber	2 %
Ash	9 %
Moisture	8 %
Vitamin A	3000 IU/kg
Vitamin D3	3000 IU/kg
Vitamin E	150 IU/kg

Table 9.3.- Technical specifications for Kaldnes™ filter media (http://www.W-M-T.com)

Specifications	
Material	Polyethylene
Specific surface area	500 m^2/m^3
Maximum fill	up to 70%
Weight per m^3	152 kg/m^3
Number of units per m^3	1,029,000
Surface per unit	4.86 cm^2
Percentage of hollow space	93%
Color	natural white

Kaldnes™ has a density of 0.96 kg/L (Helm et al., 1994; Rusten et al., 1995).

Table 9.4.- Rauschert's Biolox and Bioflow rings. These rings can be supplied with material densities adjusted from 0.95 - 1.15 g/cm^3. Manufacturing material could be PP; PE; PE, black; PE / PP, Recycled. SG = Specific gravity. (http://www.rauschertus.com/process_technologies/biological.html).

Media Type	Media Size		Media Weight		Specific Surface Area		Void Space	SG
	in.	mm	lb / ft^3	kg / m^3	ft^2 /ft^3	m^2 /m^3	%	
Bioflow 9	3/8	9	10.6	170	261	855	85	1.15
Biolox 10	3/8	10	11.2	180	195	640	82	1.09

The biofilter tank used to hold the biomedia was 208 cm in length, 56 cm in width, and 40 cm in water depth (466 L). The biomedia was enclosed in a volume within the tail section of the biofilter tank by a 2 mm pore size screen (56 x 56 cm) positioned perpendicular to the water flow and at 122 cm from the effluent area (273 L). The biomedia mixture had a volume of 140 L, hence it occupied 51.3 % of the enclosed volume section.

The filter was operated as a submerged moving bed biofilter. Water with a minimum dissolved oxygen level of 5 mg/L flowed into the biofilter. Strong aeration was provided under the biomedia mixture from air blowing through six airstones (model ASI-30, Aquatic Ecosystems). The recorded dissolved oxygen concentration in the biofilter effluent water was ~6.5 mg O_2/L.

9.2.4.- Measurements of water quality

Water samples were taken from the head of the biofilter tank, immediately after the water was filtered through the bag filter. The TAN was measured with Hach™ reagents by the salycilate method, which is specific for seawater samples (Hach™ method 8155). TAN was measured using a Hach™ Odissey spectrophotometer (model DR/2500). Readings of pH were taken with a Fisher Scientific Accumet pHmeter (model 50). Alkalinity was measured by titration (La Motte Chemical test kit, model WAT-DR). Salinity was measured with a YSI SCT meter (model 33). TAN, pH, alkalinity, salinity, and temperature were monitored

daily at 9:00 h before fish feeding. Sodium bicarbonate was added daily at 9:00 h and 17:00 h to maintain alkalinity.

9.2.5.- TAN and urea-N excretion by California halibut juveniles

The TAN and urea-N excretion rates by California halibut juveniles were estimated using the following equations, which are valid for fish weighing between 4 and 200 g (Chapter VII):

$$r_{TAN} = 115.73\ W^{-0.0453} \qquad \text{(Eq. 9.2)}$$

$$r_{UREA\text{-}N} = 16.253\ W^{-0.0108} \qquad \text{(Eq. 9.3)}$$

where r_{TAN} = rate of TAN excretion by the fish (mg TAN/kg d)

$r_{UREA\text{-}N}$ = rate of urea-N excretion by the fish (mg urea-N/kg d)

W = fish average mass (g)

For the purpose of this analysis, urea-N was assumed to be totally hydrolyzed to TAN; hence total nitrogen (TN) excreted refers to TAN plus urea-N excreted by the California halibut juveniles being reared.

9.2.6.- TAN nitrification estimated by mass balance calculations on alkalinity depletion

Nitrification requires about 0.14 eq of alkalinity for the complete oxidation of 1 g of TAN (Timmons et al., 2001). Alkalinity was maintained by daily additions of sodium bicarbonate. As stated above, alkalinity was measured daily in the

morning (~10:00 h). Therefore it was possible to estimate the rate of nitrification from alkalinity mass balance assuming that the only consumption of alkalinity was by nitrification and the only source was the sodium bicarbonate added to the system.

The mass balance of alkalinity (ALK) applied to the recirculating aquaculture system with a constant volume, was used to estimate the alkalinity depletion rate, which can be described by:

$$\frac{dC}{dt}V = Q_i(C_i - C_e) + r_{added}V - r_{depleted}V \qquad \text{(Eq. 9.4)}$$

where dC/dt = change in ALK concentration in the recirculating aquaculture system over a given period of time (meq / L d)

V = recirculating aquaculture system volume (L)

Q_i = flow rate of make-up water (L/d)

C_i = ALK concentration in system make-up water (meq / L)

C_e = ALK concentration in system effluent water (meq / L)

r_{added} = rate of ALK added to the system (meq / L d)

$r_{depleted}$ = net rate of ALK depletion (meq / L d)

The model did not discriminate between alkalinity losses that might take place in other sections of the recirculating aquaculture system and those due to the

nitrification occurring in the biofilter itself. The influent and effluent ALK concentration were considered negligible. Sodium bicarbonate (Arm & Hammer® baking soda) was used as the alkalinity source. The rate of ALK added to the system was between 0.23 and 0.28 meq/L d. Hence the following expression was used to estimate ALK depletion due to nitrification within the RAS:

$$r_{depleted}V = r_{added}V - \frac{dC}{dt}V \qquad \text{(Eq. 9.5)}$$

where

$$\frac{dC}{dt} = \left(\frac{C_t - C_0}{t_t - t_0}\right) \qquad \text{(Eq. 9.6)}$$

where, C_t = ALK concentration (meq / L) in system water at time t (t_t, d)

C_0 = ALK concentration (meq / L) in system water at time 0 (t_0, d)

Hence the following expression was used to estimate TAN nitrified based on ALK depletion:

$$TAN_{ALK} = \frac{r_{depletion}V}{0.14} \qquad \text{(Eq. 9.7)}$$

where, TAN_{ALK} = TAN consumed by nitrification based on alkalinity depletion (mg/d)

9.2.7.- TAN nitrification estimated by mass balance calculations

Following a methodology described by several authors (Imsland et al., 1995; Eikebrokk and Piedrahita, 1997; Thomas and Piedrahita, 1997; Fivelstad et al., 1999), the nitrification rate was determined by mass balance analysis under farm-like conditions for the complete recirculating aquaculture system. Mass balance analysis for the entire system was performed for those dates in which all fish held in the recirculating aquaculture system were weighed and counted. The fish were counted and weighed approximately every two weeks, between January 14 and April 15 on 2003.

The mass balance of TAN applied to the recirculating aquaculture system with a constant volume, was used to estimate the nitrification rate, which can be described by (Eikebrokk and Piedrahita, 1997; Thomas and Piedrahita, 1997):

$$\frac{dC}{dt}V = Q_i(C_i - C_e) + r_{diff}V + r_xV - r_yV \qquad \text{(Eq. 9.8)}$$

where dC/dt = change in TAN concentration in the recirculating aquaculture system over a given period of time (mg / L d)

V = recirculating aquaculture system volume (L)

Q_i = flow rate of make-up water (L/d)

C_i = TAN concentration in system make-up water (mg / L)

C_e = TAN concentration in system effluent water (mg / L)

r_{diff} = rate of diffusion of TAN between water and the atmosphere (mg / L d)

r_x = net rate of TN excreted by the fish in the recirculating aquaculture system (mg / L d)

r_y = net rate of TAN nitrified (mg / L d)

The model did not discriminate between nitrification that might take place in other sections of the recirculating aquaculture system and the nitrification occurring in the biofilter itself. For the purposes of the present study, it was assumed that all nitrification occurred in the biofilter, thus only biofilter media volume and biofilter surface area were used for nitrification rates estimates. The recirculating aquaculture system was assumed to be under steady state conditions for a 24 h period, hence mass balances were made for steady state conditions. It was also assumed that the only source of TAN was fish TAN excretion. The rate of diffusion of TAN within the liquid film was estimated to be negligible, based on observations by Thomas and Piedrahita (1998). The effluent TAN concentration only considered TAN, since urea-N was not sampled. Hence the following expression was used to estimate TN nitrified by the biofilter:

$$TAN_{MB} = r_y V = r_x V - (Q_i C_e) \qquad \text{(Eq. 9.9)}$$

where

$$r_x V = (r_{TAN} + r_{UREA-N}) * B \qquad \text{(Eq. 9.10)}$$

where, TAN_{MB} = TAN consumed by nitrification (mg/d)

B = total fish biomass (kg)

9.2.8.- Biofilter performance

Assuming that the nitrification process occurs entirely in the biomedia, the actual biofilter performance was estimated from a calculation of the volumetric nitrification capacity (VNR, g TN consumed/m^3 media per day) and the surface nitrification capacity (SNR, g TN consumed/m^2 media per day) (Timmons et al., 2001):

$$VNR = \frac{TAN_X}{V} * a \qquad \text{(Eq. 9.11)}$$

$$SNR = \frac{TAN_X}{S} * a \qquad \text{(Eq. 9.12)}$$

where, TAN_X = TAN_{ALK} or TAN_{MB} consumed by nitrification (mg/d)

V = volume of media in the biofilter (m^3)

S = total surface area of the media in the biofilter (m^2)

a = conversion factor (1 g / 1000 mg)

Finally, the hydraulic loading rate (HLR, m^3/m^2 d) (Timmons et al., 2001) is expressed as:

$$HLR = \frac{Q}{W * D} \qquad \text{(Eq. 9.13)}$$

where, Q = volume of water pumped through the biofilter (m^3/d)

W = biofilter width (m)

D = biofilter depth (m)

9.3.- RESULTS

9.3.1.- Total nitrogen excretion as TAN and Urea-N

Production rates of TAN, urea-N, and TN calculated from Eq. 9.2 and Eq. 9.3, are presented in Table 9.5. The TN was observed to increase over time as the fish grew. At the time of the analysis, an attached experiment was set up, called "density", which was terminated prior April 15, 2003 (Table 9.5). System volume remained unchanged for the April 15, 2003 test.

9.3.2.- TAN nitrification estimated by alkalinity depletion

Sodium bicarbonate was added twice a day to the recirculating aquaculture system. Estimated TAN_{ALK} nitrified, based on the amount of alkalinity consumed, was between 2570 and 8568 mg/d (Table 9.6).

Table 9.5.- Production rates of TAN, urea-N, and TN calculated from Eq. 9.2 and Eq. 9.3. System volume is 3000 L. Density refers to the density experiment (SRC in Fig. 9.2).

		Raceway				
Date	14-Jan-03	1	2	3	4	Density
	Fish #	616	544	392	214	264
	Total biomass, g	6795	10539	4579	2404	2980
	Average biomass, g	11.0	19.4	11.7	11.2	11.3
	r_{TAN}, mgTAN/kg d	104	101	104	104	104
	r_{Urea-N}, mg Urea-N/kg d	16	16	16	16	16
	mg TAN/d	705	1066	474	249	309
	mg Urea-N/d	108	166	72	38	47
	TN mg/d	3236				
Date	30-Jan-03	1	2	3	4	Density
	Fish #	609	541	391	214	264
	Total biomass, g	8069	12443	5744	3135	3508
	Average biomass, g	13.3	23.0	14.7	14.7	13.3
	r_{TAN}, mgTAN/kg d	103	100	102	102	103
	r_{Urea-N}, mg Urea-N/kg d	16	16	16	16	16
	mg TAN/d	831	1249	589	321	361
	mg Urea-N/d	128	196	91	49	55
	TN mg/d	3870				
Date	15-Feb-03	1	2	3	4	Density
	Fish #	602	530	390	213	264
	Total biomass, g	9114	15274	6930	3721	4160
	Average biomass, g	15.1	28.8	17.8	17.5	15.8
	r_{TAN}, mgTAN/kg d	102	99	102	102	102
	r_{Urea-N}, mg Urea-N/kg d	16	16	16	16	16
	mg TAN/d	933	1518	704	378	425
	mg Urea-N/d	144	239	109	59	66
	TN mg/d	4575				

Table 9.5.- (continuation)

		Raceway				
Date	1-Mar-03	1	2	3	4	Density
	Fish #	588	525	389	210	262
	Total biomass, g	10700	16274	7972	3323	4836
	Average biomass, g	18.2	31.0	20.5	15.8	18.5
	r_{TAN}, mgTAN/kg d	101	99	101	102	101
	r_{Urea-N}, mg Urea-N/kg d	16	16	16	16	16
	mg TAN/d	1086	1612	805	339	490
	mg Urea-N/d	169	255	125	52	76
	TN mg/d	5010				
Date	15-Apr-03	1	2	3	4	
	Fish #	441	467	470	530	
	Total biomass, g	13544	15176	15035	18765	
	Average biomass, g	30.7	32.5	32.0	35.4	
	r_{TAN}, mgTAN/kg d	99	99	99	98	
	r_{Urea-N}, mg Urea-N/kg d	16	16	16	16	
	mg TAN/d	1342	1500	1487	1848	
	mg Urea-N/d	212	238	235	293	
	TN mg/d	7156				

Fish from the density experiment were distributed into the raceways prior to April 15.

System volume did not change on April 15.

Table 9.6.- Estimation of TAN nitrified through the consumption of alkalinity (TAN_{ALK}). The Fish weighing column stands for the date on which fish were weighed. Alkalinity (second column) stands for the morning reading value before the addition of $NaHCO_3$ (third column). Alkalinity at 0h stands for the calculated alkalinity value after the $NaHCO_3$ addition. Alkalinity at 24h stands for alkalinity measured the next day, before $NaHCO_3$ addition. Alkalinity 24h – 0h stands for the alkalinity change between time 0h and 24h. The alkalinity 24h - 0h was used to determine the amount of TAN_{ALK} nitrified. System volume was 3000 L.

Fish weighing	Alkalinity	$NaHCO_3$ added	Alkalinity Added	Alkalinity at 0h	Alkalinity at 24h	Alkalinity 24h - 0h	TAN_{ALK} nitrified
	meq/L	g	meq	meq/L	meq/L	meq/L	mg/d
14-Jan-03	2.40	60	714	2.64	2.52	0.12	2570
29-Jan-03	2.48	60	714	2.72	2.56	0.16	3427
15-Feb-03	2.64	60	714	2.88	2.60	0.28	5998
1-Mar-03	2.20	70	834	2.48	2.28	0.20	4284
15-Apr-03	3.60	60	714	3.84	3.44	0.40	8568

9.3.3.- TAN nitrification estimated by mass balance calculations

The TAN_{MB} consumption rates by the submerged biofilter calculated from mass balance for the entire recirculating system are presented in Table 9.7. The TAN_{MB} nitrification rates by the biofilter increased with TN excreted by the fish. Estimated TAN_{MB} nitrified was between 3233 and 7154 mg/d (Table 9.7).

9.3.4.- Biofilter performance

Hydraulic loading rate was kept constant during the experimental period (Table 9.8). Volumetric nitrification capacity (VNR) and surface nitrification capacity (SNR) calculated either for TAN_{ALK} or TAN_{MB} increased with the fish biomass. Maximum VNR was 61 g/m^3 d for TAN_{ALK} and 51 g/m^3 d for TAN_{MB} (Table 9.8). Maximum SNR was 0.10 g/m^2 d for TAN_{ALK} and 0.09 g/m^2 d for TAN_{MB} (Table 9.8).

9.4.-DISCUSSION

The removal of TAN is one of the most important factors in maintaining water quality and fish health conditions in recirculating fish cultures. To design effective closed recirculating systems, it is important to determine the nitrification capacity of biological filters. Recently, a new variation of the submerged bed biofilter technology has been introduced, in which the traditional fixed media is replaced by a moving media. A submerged

Table 9.7.- Estimation of TAN nitrified by the submerged moving bed biofilter determined by mass balances for the entire system (TAN_{MB}). The "Fish weighing" column stands for the date on which fish were weighed. The weight obtained that day was used to calculate TN production (Eq 9.2, Eq. 9.3, and Eq. 9.6). The "Sampling date" column stands for the day when water was sampled for TAN. Recirculating system volume was 3000 L.

Fish weighing	Fish biomass kg	TN excreted mg/d	Make up water L/d	Sampling date	TAN_out mg/L	TAN_{MB} nitrified mg/d
14-Jan-03	27	3236	30	18-Jan-03	0.07	3233
29-Jan-03	33	3870	30	27-Jan-03	0.1	3867
15-Feb-03	39	4575	30	9-Feb-03	0.36	4564
1-Mar-03	43	5010	30	20-Mar-03	1.04	4979
15-Apr-03	63	7156	30	10-Apr-03	0.04	7154

Table 9.8.- Submerged moving bed biofilter performance. The hydraulic loading rate was constant for the time of the experiment at 0.13 m^3/m^2 d. Make-up water was 0.03 m^3/d.

	TAN_{ALK}		TAN_{MB}	
Fish weighing	VNR g/m^3 d	SNR g/m^2 d	VNR g/m^3 d	SNR g/m^2 d
14-Jan-03	18	0.03	23	0.04
29-Jan-03	24	0.04	28	0.05
15-Feb-03	43	0.07	33	0.06
1-Mar-03	30	0.05	36	0.06
15-Apr-03	61	0.10	51	0.09

Media volume: 0.14 m^3; Media surface area:82.3 m^2.

moving bed biofilter was used to remove TAN from the recirculating system used to grow California halibut (Appendix A.1). There are few reports on nitrification rates achieved by a submerged moving bed in aquaculture recirculating systems (Zhu and Chen, 1999; Lekang and Kleppe, 2000).

In the present research nitrification rates estimated for the submerged moving bed biofilter were based on alkalinity depletion and mass balances for the whole recirculating system and not for the biofilter alone. Therefore, nitrification taking place by nitrifying bacteria attached to the recirculating system walls is assigned to the biofilter. In addition all TAN sources are attributed to the fish being reared in the recirculating system. Finally it was assumed that urea-N excreted by the fish was hydrolyzed immediately to TAN.

Kikuchi et al. (1994) reported a linear relationship between the amount of ammonia oxidized and a decrease in alkalinity, which allowed them to predict that the alkalinity of a recirculating seawater system loaded with fish (alkalinity of natural seawater is ~2.2-2.5 meq/L) would be mostly depleted when 20 mg TAN/L were nitrified. Lu and Piedrahita (1993) reported a drop in alkalinity from 3 to 0.4 meq/L after 143 days when testing the nitrification performance of trickling filters. Alkalinity fluctuations due to nitrification were also reported by Sandu et al. (2002) while studying fluidized bed filter performance. Therefore an estimation of the TAN nitrified by a biofilter in a recirculating aquaculture system can be determined by knowing the alkalinity consumed by the nitrification process. In

this research, alkalinity was added daily, between 595 and 1429 meq/d (0.19 and 0.48 meq/L d), to keep up with its consumption by nitrification.

The TN excretion (TAN and urea-N) for juvenile California halibut was calculated using Eq. 9.2 and Eq. 9.3. The theoretical nitrification rate determined from alkalinity changes (TAN_{ALK}) (0.14 meq/mg TAN) (Table 9.6) and that calculated from TN excretion (TAN_{MB}) (Table 9.7) were similar. These results are in agreement with findings of Kikuchi et al. (1994) where nitrification also was related with alkalinity.

The TAN_{ALK} (Table 9.6) and TAN_{MB} (Table 9.7) nitrification estimated for a submerged moving bed biofilter increased over time as fish biomass increased. California halibut juveniles were fed at a fixed ratio of 1.2% body mass, therefore more feed was given to the largest fish. TAN excretion by fish is related to the amount of protein content in the daily feed eaten (Clark et al., 1985; Brunty et al., 1997; Carter et al., 1998; Engin and Carter, 2001). Due the nature of the present research, it was not possible to determine the maximum nitrification capacity of the submerged moving bed biofilter studied.

The largest VNR values calculated (Eq. 9.7) were 61 g/m^3 d for TAN_{ALK} and 51 g/m^3 d for TAN_{MB} nitrification. The largest SNR (Eq. 9.8) values calculated were 0.10 g/m^2 d for TAN_{ALK} and 0.09 g/m^2 d for TAN_{MB} nitrification. The VNR and SNR values obtained in the present research were lower than the ones reported

by other authors. Lu and Piedrahita (1993) reported nitrification rates with a range of 0.05 to 0.15 g/m^2 d for trickling filters. Malone et al. (1993) reported TAN conversion rates, at a demonstration facility, of 0.28 g/m^2 d (41 g/m^3 d) for an RBC and 0.29 g/m^2 d (308 g/m^3 d) for a mechanically-washed bead filter. Westerman et al. (1996) reported TAN conversion rates, at commercial tilapia facility, of 0.25 g/m^2 d (101 g/m^3 d) for an RBC and about 0.10 to 0.15 g/m^2 d (122 to 318 g/m^3 d) for the other biofilters tested (upflow sand filter, fluidized bed sand filter, and floating bead filter). Losordo et al. (2000) reported TAN conversion rates at a pilot facility (designed to yield 45 metric tons of tilapia) of 0.43 g/m^2 d for a trickling biofilter.

It has been reported that nitrification rates in a biofilter depend on TAN concentration and diffusion rates within the biofilm boundaries (Timmons et al., 2001). Aquaculture recirculating systems are operated at very low TAN concentrations. Zhu and Chen (1999) found that a minimum TAN concentration of 0.07±0.05 mg/L was needed to support a steady-state nitrification biofilm in a submerged moving bed with Kaldnes™ media. In the present research water system TAN concentrations were between 0.04 and 1.04 mg/L (Table 9.7). Zhu and Chen (2003) found that inducing turbulent flow by air diffusion had a substantial effect on the nitrification rate at low hydraulic loading rates mainly because of the improvement of the hydraulic conditions for mass (oxygen and TAN) transfer between the bulk water and the biofilm surface. In the present research strong aeration was supplied by seven airstones located on the bottom

of the biofilter tank. High air flow rates might cause greater shearing forces, which reduce the biofilm thickness (Zhu and Chen, 2001) increasing diffusion rates (Zhu and Chen, 2003). According to Zhu and Chen (2001), a higher Reynolds number (Re = 66710) led to a TAN removal five times higher than occurred when a small Reynolds number (Re = 1668) was used.

The biomedia tested in this research always had a light brown coloration. The light brown coloration is presumably from bacterial biofilm growth. Lu and Piedrahita (1993) reported an increase in biofilm formation and nitrification rate when trickling biofilters were operated at a higher loading rate (maximum ~3765 m^3/m^2 d), which also resulted in clogging of the trickling filter. Submerged moving bed biofilters are promoted as self-cleaning devices, since excess of biofilm is removed from the media by the water turbulence caused by aeration (Odegaard et al., 1994; www.kmt.no). Due to the nature of this study, the effects of hydraulic loading rate, TAN loading rate, and air diffusion on biofilm formation and nitrification rates were not investigated.

9.5.- CONCLUSIONS

The main conclusions of the present chapter are:

a) Theoretical nitrification rates determined from alkalinity changes (TAN_{ALK}) and that calculated from TN excretion (TAN_{MB}) were similar. Hence, nitrification is closely related to alkalinity.

b) Under the scenario studied, the largest VNR and SNR for the submerged moving bed biofilter were 61 g/m^3 d and 0.10 g/m^2 d, respectively.

c) The maximum nitrification achievable by the submerged moving bed biofilter analyzed here was not determined in this research.

9.6.- FURTHER RESEARCH

Further studies are recommended in the following areas:

- Zhu and Chen (2003) reported that an increase in turbulence of the fluid in contact with the biofilter would maximize the TAN removal. In the present research the submerged moving bed biofilter operated under strong aeration and short hydraulic residence time. The performance of the biofilter described in this research under varying scenarios of aeration and residence time should be studied.

- The combined effects of hydraulic loading rate, TAN loading rate, and air diffusion on biofilm formation in the SMBB and nitrification rates should be studied.

CHAPTER X

BIOENGINEERING DESIGN OF A RECIRCULATING AQUACULTURAL FACILITY FOR CALIFORNIA HALIBUT GROWOUT

10.1.- INTRODUCTION

A recirculating pilot facility was designed to grow California halibut from 50 to 1000 g. The design was performed with the bio-engineering data determined in the present work. Most bio-engineering data (relative swimming velocity, stocking density, oxygen consumption, and TAN and urea-N excretion) were determined for fish up to 200 g. As no data were available for larger fish, these data were projected for fish up to 1000 g, introducing uncertainty in the design.

The recirculating system will be built at the Bodega Marine Laboratory. Knowledge gained through the design and operation of this system will provide an initial milestone for the commercial culture of California halibut. The goals of this recirculating system are to test the bio-engineering data gathered in the present research, and to provide a pilot system in which more bio-engineering data can be gathered.

10.2.- SITE DESCRIPTION

The Recirculating California Halibut Facility will be built at the University of California's Bodega Marine Laboratory. The area available at the Bodega Marine Laboratory for the pilot recirculating facility is 10.8 m by 6.7 m. A schematic diagram of the proposed system is shown in Fig. 10.1. Four fish culture tanks will be used and the effluent from each tank will flow into a swirl separator for the immediate removal of the heaviest particles. The effluents overflowing the swirl separators will converge into a single pipe, which will deliver the water to a drum filter with a 60 μm mesh. The drum filter will be followed by a submerged moving bed biofilter. Within the biofilter, the water will be heated and pumped back to the head of the culture tanks. Packed columns with pure oxygen will be used in each tank. Pure oxygen will be produced on site by an oxygen generator.

10.3.- CULTURE UNIT

Raceways and circular shapes are the two most common fish tanks used in aquaculture facilities. Circular tanks, in comparison to raceways, have a more uniform water quality, independence between flow rate and water velocity, and less tendency to accumulate solids (Timmons et al., 2001). Raceways make better use of floor space, but usually have significant water quality gradients from the influent to the effluent.

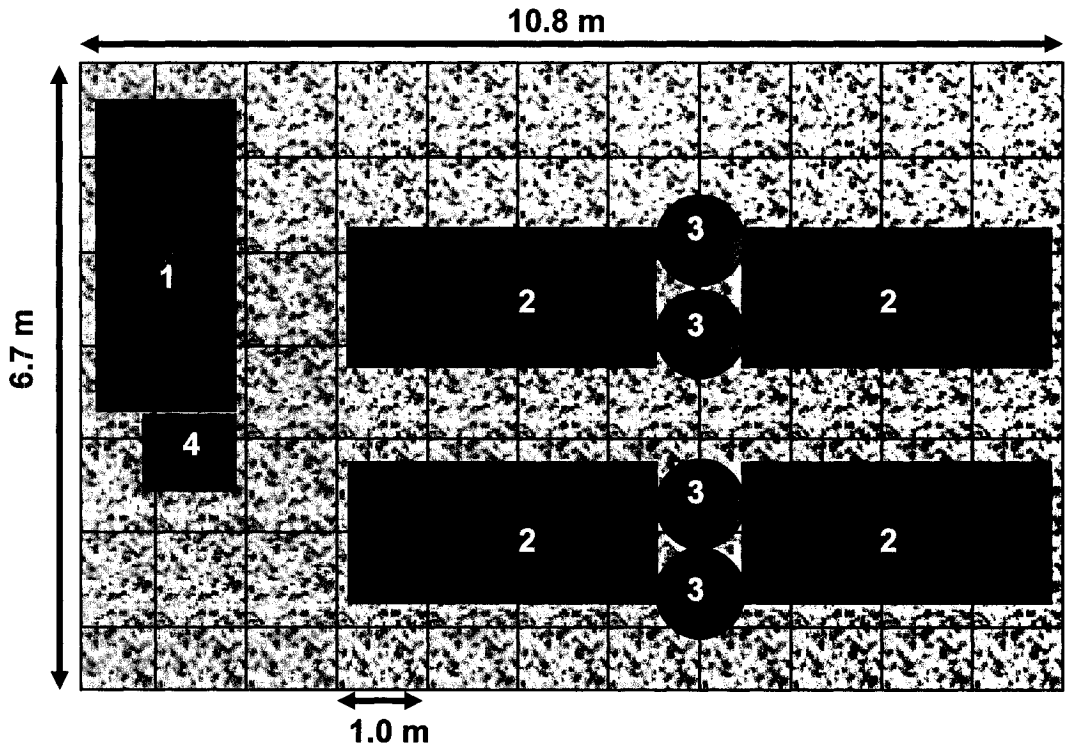

Figure 10.1.- Schematic diagram of the proposed Recirculating Pilot Facility at the Bodega Marine Laboratory. 1: biofilter/sump; 2: fish tank; 3: swirl separator; 4: drum filter

The Burrows tank (Burrows and Chenoweth, 1970) is a modified raceway with circular flow around a longitudinal central septum and two floor drains. It acts as a circulating tank while still retaining some characteristics of a rectangular tank. A substantially modified version of this tank shape was selected for the pilot facility. Tank dimensions were chosen to be 1.5 m width, 3.0 m length and 0.76 m height (Fig. 10.2). A partial wall will split the tank in two. The tank will have two drains (each ~76 mm or 3.0 inches in diameter).

10.4.- NUMBER OF FISH TO BE PRODUCED

The results for juvenile California halibut suggest that the stocking density effect becomes evident after a certain threshold level (Chapter V). It seems that initial stocking densities of 100% PCA will give a better growth over time than the other stocking densities tested (200 and 300% PCA). The fish stock must be split to reduce fish biomass per area when stocking density is close to 200% PCA, otherwise a significant SGR (specific growth rate) reduction is to be expected. A general equation for stocking density, in biomass per surface area is:

$$BFA = \beta * \left(\frac{W}{TSA} \right) * \kappa \qquad \text{(Eq. 10.1)}$$

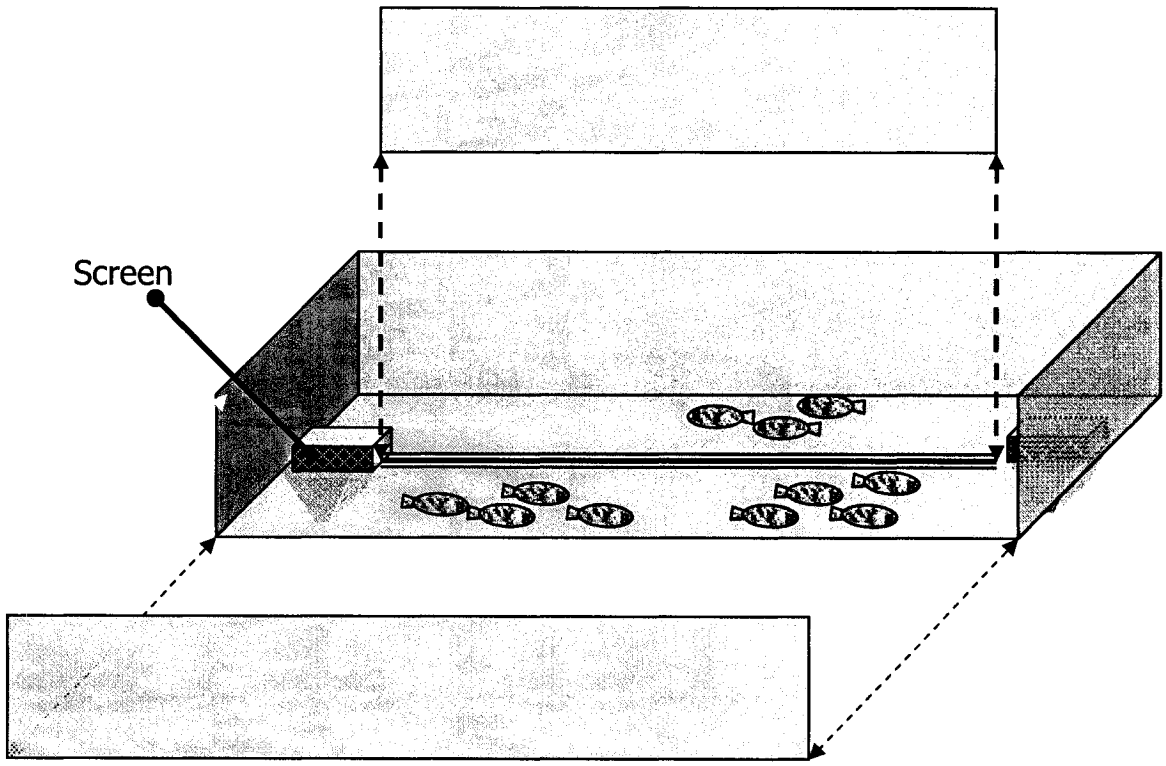

Figure 10.2.- Schematic diagram of the rectangular culture tank. For clarity, one wall has been pulled out, and the divisional wall has been lifted.

where BFA = stocking density (kg/m^2)

β = stocking index (ie. 1 for 100%; 2 for 200%)

W = fish average biomass (g)

TSA = fish average total surface area (cm^2)

κ = conversion factor (10 kg cm^2 g^{-1} m^{-2})

The BFA can be determined by using the relationships between TSA and fish mass (Appendix A.2, Fig. A2.8) valid for fish weighing 66 to 270 g:

$$TSA = 11.224 * W^{0.5716} \qquad \text{(Eq. 10.2)}$$

The number of fish per square meter of bottom tank area (NFA) can be determined by:

$$NFA = \frac{c}{TSA} \qquad \text{(Eq. 10.3)}$$

where, c = conversion factor (10000 cm^2/m^2).

The fish length (TL, cm) corresponding to a given fish surface area can be determined from (Appendix A.2, Fig. A2.7, valid for fish weighing between 0.77 and 270 g):

$$TL = 1.7589 * TSA^{0.5076} \qquad \text{(Eq. 10.4)}$$

Maximum water velocity and biomass stocking values for the intensive culture of California halibut weighing between 50 and 1000 g are shown in Table 10.1. These reference values allow for a rapid determination of the minimum (100% PCA) and maximum (200% PCA) stocking densities recommended for a given fish size. In addition the maximum water velocity to achieve a relative swimming velocity of 1 bl/s is also tabulated (Chapter IV). Finally, a recommended pellet size is also tabulated for a given fish size.

The four tanks to be used to rear California halibut to commercial size have a surface area available for culture of 4.5 m^2 each, which will allow up to ~620 fish of 1000 g in body mass to be held (Table 11.2). Although the four culture tanks can hold up to ~3430 California halibut of 50 g, based on a 200% PCA, it is not necessary to raise this number of fish from the beginning (Table 11.2). At the time the fish reach 1000 g, about ~620 fish can be held at a maximum density of 200% PCA. Therefore, the initial stocking density for the entire system will be ~ 682 fish if a 10% mortality is assumed.

10.5.- WATER FLOW WITHIN THE CULTURE UNIT

Water flow will be estimated with the assumption that the Recirculating Pilot system is stocked initially with 682 fish. Also a mortality of 10% from the stock at 50 g until they reach 1000 g will be assumed. Hence, the final total population at harvest time will be ~620 fish (Table 10.2).

Table 10.1.- Stocking values for the intensive culture of California halibut. Densities of 100% PCA and 200% PCA are indicated for the biomass of fish per bottom tank area (BFA) or the number of fish per bottom tank area (NFA). Maximum water velocity (IV) in the culture tank corresponds to a relative swimming velocity of 1 bl/s.

			100% PCA		200% PCA			
Fish size	TL	TSA	BFA	NFA	BFA	NFA	V	Feed size
g	cm	cm^2	kg/m^2	$fish/m^2$	kg/m^2	$fish/m^2$	cm/s	mm
50	19	105	5	95	10	190	19	3
100	23	156	6	64	13	128	23	5
150	26	197	8	51	15	102	26	5
200	28	232	9	43	17	86	28	8
250	30	264	9	38	19	76	30	8
300	32	292	10	34	21	68	32	10
350	33	319	11	31	22	63	33	10
400	35	345	12	29	23	58	35	10
450	36	369	12	27	24	54	36	10
500	37	392	13	26	26	51	37	10
550	38	414	13	24	27	48	38	10
600	39	435	14	23	28	46	39	10
650	40	455	14	22	29	44	40	10
700	41	475	15	21	29	42	41	10
750	41	494	15	20	30	41	41	10
800	42	512	16	20	31	39	42	10
850	43	530	16	19	32	38	43	10
900	44	548	16	18	33	36	44	10
950	44	565	17	18	34	35	44	10
1000	45	582	17	17	34	34	45	10

TL (cm) = 1.7589 * TSA $(cm^2)^{0.5076}$, r^2 = 0.9976 for 0.77 to 270 g California halibut. (Appendix A.2, Fig. A2.7)

TSA (cm^2) = 11.224 * W $(g)^{0.5716}$, r^2 = 0.9074 for 66 to 270 g California halibut (Appendix A.2, Fig. A2.8).

Table 10.2.- Minimum and maximum fish stocking density per culture tank with 4.5 m^2 of bottom area. "Maximum fish" column stands for the total number of fish that can be reared within the four culture tanks, assuming a maximum final stocking density of 200% PCA.

Fish size	Min. stocking per tank 100%		Max. stocking per tank 200%		Maximum
g	kg	Fish	kg	fish	fish
50	21.4	428	42.8	857	3428
100	28.8	288	57.7	577	2307
150	34.3	229	68.6	457	1829
200	38.8	194	77.6	388	1552
250	42.7	171	85.4	342	1366
300	46.2	154	92.3	308	1231
350	49.3	141	98.6	282	1127
400	52.2	131	104.4	261	1044
450	54.9	122	109.8	244	976
500	57.5	115	114.9	230	919
550	59.8	109	119.7	218	870
600	62.1	104	124.2	207	828
650	64.3	99	128.6	198	791
700	66.4	95	132.7	190	758
750	68.4	91	136.7	182	729
800	70.3	88	140.5	176	703
850	72.1	85	144.2	170	679
900	73.9	82	147.8	164	657
950	75.6	80	151.3	159	637
1000	77.3	77	154.6	155	619

Bio-engineering data presented in the previous chapters will provide the information required to estimate the bio-engineering parameters for the Recirculating Pilot system design. The worst case scenario will consist of having the tanks loaded at maximum, which is at 200% PCA (Table 10.3).

Knowing the feed requirements, it was possible to determine oxygen consumption, TAN excretion, and urea-N excretion per day per unit of feed eaten by the fish (Table 10.3). Oxygen consumption per unit of feed eaten was determined by averaging the maximum daily oxygen consumption for fish weighing between 110 and 166 g (Chapter VI, Table 6.3): 0.77 g O_2/g feed. Total ammonia nitrogen (TAN) excretion per unit of nitrogen eaten was determined by averaging the daily TAN excretion for fish weighing between 111 and 199 g (Chapter VII, Table 7.5): 114.8 mg TAN/g N-feed. Urea as nitrogen (urea-N) excretion per unit of nitrogen eaten was determined by averaging the daily urea-N excretion for fish weighing between 111 and 199 g (Chapter VII, Table 7.5): 22.2 mg urea-N/g N-feed.

As noted above ~682 fish of 50 g each will need to be stocked in the Pilot Recirculating System. It was concluded in Chapter V that a minimum of 100% PCA and a maximum of 200% PCA are recommended to grow California halibut. Table 10.4 shows the number of tanks needed to grow the fish at stocking densities between 98 and 200% PCA as well as feed, oxygen consumption, TAN and urea-N excretion per tank per day.

Table 10.3.- Bio-engineering parameters estimated at maximum fish culture density (200% PCA) per tank.

Fish size g	200% BFA kg/m^2	200% NFA fish/m^2	Feed %BW	Feed g/m^2 d	Oxygen g/m^2 d	N-feed g/m^2 d	TAN mg/m^2 d	Urea-N mg/m^2 d
50	10	190	1	95	73	6.6	750	145
100	13	128	1	128	99	8.8	1009	196
150	15	102	1	152	117	10.5	1201	233
200	17	86	1	172	133	11.9	1358	263
250	19	76	1	190	146	13.1	1495	290
300	21	68	1	205	158	14.1	1616	313
350	22	63	1	219	169	15.1	1726	335
400	23	58	1	232	179	16.0	1828	354
450	24	54	1	244	188	16.8	1923	373
500	26	51	1	255	197	17.6	2011	390
550	27	48	1	266	205	18.3	2095	406
600	28	46	1	276	213	19.0	2175	422
650	29	44	1	286	220	19.7	2251	436
700	29	42	1	295	227	20.3	2323	450
750	30	41	1	304	234	20.9	2393	464
800	31	39	1	312	240	21.5	2460	477
850	32	38	1	321	247	22.1	2525	490
900	33	36	1	328	253	22.6	2587	502
950	34	35	1	336	259	23.1	2648	513
1000	34	34	1	344	265	23.6	2707	525

Oxygen consumption was estimated based on 0.77 g O_2/g feed.
TAN excretion was estimated based on 114.8 mg TAN/g N-feed.
Urea-N excretion was estimated on 22.2 mg urea-N/g N-feed
EWOS feed has 43% protein.

Table 10.4.- Proposed production plan per culture tank for the Recirculating Pilot system at the Bodega Marine Laboratory. A mortality of 10% while fish grow from 50 g to 1000 g and a 1%BW feeding rate were assumed.

Fish size g	Total fish #	Tanks #	Fish #/tank	PCA %	Biomass kg/tank	Feed g/tank d	N-feed g/tank d	Oxygen g/tank d	TAN mg/tank d	Urea-N mg/tank d
50	682	1	682	159	34	341	23	263	2686	521
100	679	2	339	118	34	339	23	261	2673	518
150	675	3	225	98	34	338	23	260	2659	516
200	672	3	224	115	45	448	31	345	3528	684
250	668	3	223	130	56	557	38	429	4388	851
300	665	4	166	108	50	499	34	384	3930	762
350	662	4	165	117	58	579	40	446	4562	884
400	658	4	165	126	66	658	45	507	5187	1006
450	655	4	164	134	74	737	51	568	5807	1126
500	652	4	163	142	81	815	56	627	6419	1245
550	649	4	162	149	89	892	61	687	7026	1362
600	645	4	161	156	97	968	67	745	7626	1479
650	642	4	161	162	104	1044	72	804	8221	1594
700	639	4	160	169	112	1118	77	861	8809	1708
750	636	4	159	174	119	1192	82	918	9391	1821
800	633	4	158	180	127	1265	87	974	9967	1932
850	629	4	157	185	134	1338	92	1030	10537	2043
900	626	4	157	191	141	1409	97	1085	11101	2152
950	623	4	156	196	148	1480	102	1140	11659	2261
1000	620	4	155	200	155	1550	107	1194	12211	2368

Oxygen consumption was estimated based on 0.77 g O_2/g feed. TAN excretion was estimated based on 114.5 mg TAN/g N-feed. Urea-N excretion was estimated based on 22.2 mg urea-N/g N-feed. EWOS feed has 43% protein. Biomass (kg/tank) is within the ranges for stocking density shown in Table 10.2.

10.5.1.- Water flow requirements based on oxygen

The Recirculating Pilot system will operate at a temperature of 21± 1 °C and at a salinity of 30 ± 2 g/L. Under these conditions, the air solubility of oxygen in seawater will be 7.48 mg/L (100% saturation) (LeRoy, 1993). For design purposes, the water entering the rearing unit will be saturated with oxygen at 150% (~11.22 mg/L). The target for dissolved oxygen leaving the tank will be no less than 5 mg/L (~67% saturation). A mass balance was performed to estimate the water flow required per tank (Table 10.5). Steady state conditions were assumed for the mass balance. It was assumed that no oxygen was produced within the culture unit or lost or gained across the water surface. Hence:

$$Q_{TANK} = \frac{\text{Consumption}}{C_{IN} - C_{OUT}} * d \qquad \text{(Eq. 10.5)}$$

where, Q_{TANK} = is the tank flow (m^3/h)

Consumption = oxygen consumed as shown in Table 10.4 (g/tank d)

C_{IN} = dissolved oxygen entering (mg/L)

C_{OUT} = dissolved oxygen leaving (mg/L)

d = factor to convert units (mg m^3 d/24 g L h)

Once the flow rate per tank (Q_{TANK}) was determined, the velocity of the water flowing within the culture unit was also calculated (Table 10.5). The velocity was calculated for one of the two tank sections (tank was evenly divided by a partial

Table 10.5.- Tank flow (Q_{TANK}) and total system flow (Q_{TOTAL}) estimated by mass balance on oxygen requirements. TL: fish total length; V: water velocity within the culture tank.

Fish size g	TL cm	Q_{TANK} m^3/h	V cm/s	Tanks #	Q_{TOTAL} m^3/h
50	19	1.8	0.22	1	1.8
100	23	1.8	0.22	2	3.5
150	26	1.7	0.22	3	5.2
200	28	2.3	0.29	3	6.9
250	30	2.9	0.36	3	8.6
300	32	2.6	0.32	4	10.3
350	33	3.0	0.37	4	12.0
400	35	3.4	0.43	4	13.6
450	36	3.8	0.48	4	15.2
500	37	4.2	0.53	4	16.8
550	38	4.6	0.58	4	18.4
600	39	5.0	0.62	4	20.0
650	40	5.4	0.67	4	21.5
700	41	5.8	0.72	4	23.1
750	41	6.2	0.77	4	24.6
800	42	6.5	0.82	4	26.1
850	43	6.9	0.86	4	27.6
900	44	7.3	0.91	4	29.1
950	44	7.6	0.96	4	30.5
1000	45	8.0	1.00	4	32.0

wall). Velocity for the tank section was calculated as the average tank velocity by using the continuity equation:

$$V = \frac{Q}{A} = \frac{Q_{TANK}}{W * D} * k \qquad \text{(Eq. 10.6)}$$

where, V = uniform water velocity within the tank section (cm/s)

Q = water flow within the tank (m^3/h)

A = tank section cross sectional area (m^2)

W = tank section width (m)

D = tank water depth (m)

k = conversion factor (cm h/36 m s)

The partial wall divider was assumed to have a thickness of 0.02 m, therefore the tank section width will be 0.74 m. Water depth was assumed to be 0.3 m. The water velocity calculated was well below the maximum water velocity tolerated by California halibut (1 bl/s; Table 10.1); therefore there are no constraints to the flow rates determined based on oxygen requirements.

10.5.2.- Water flow requirements based on TAN

The Recirculating Pilot system will operate at a pH between 7.5 and 8.0. The target for TAN leaving the tanks will be no more than 4 mg/L. Under these conditions, the NH_3-N concentration will be between 0.0088 and 0.086 mg/L, which is within the range recommended as safe for flatfish species (Person Le-

Ruyet et al., 1997). For design purposes, the target for the water entering the rearing unit will be 1.0 mg TAN/L. A TAN of 1.0 mg/L entering the culture tanks was regularly monitored in the recirculating system for California halibut at the University of California, Davis. A mass balance was performed to calculate the water flow required per tank and for the entire recirculating system (Table 10.6). Steady state conditions were assumed for the mass balance. It was also assumed that no TAN was consumed within the culture unit and that the urea-N excreted would be converted to TAN; hence the production term included TAN plus urea-N (Table 10.4). Hence:

$$Q_{TANK} = \frac{Production}{C_{N-OUT} - C_{N-IN}} * m \qquad \text{(Eq. 10.5)}$$

where, Q_{TANK} = is the tank flow (m^3/h)

Production = TAN and urea-N excretion as shown in Table 10.4 (mg/tank d)

C_{N-IN} = TAN and urea-N concentration entering (mg/L)

C_{N-oUT} = TAN and urea-N concentration leaving (mg/L)

m = factor to convert units (m^3 d/24000 L h)

Table 10.6.- Tank flow (Q_{TANK}) and total system flow (Q_{TOTAL}) estimated by mass balance on TAN requirements. TL: fish total length; V: water velocity within the culture tank. Influent TAN was considered to be 1 mg/L, and effluent TAN to be 4 mg/L.

Fish size g	TL cm	Q_{TANK} m^3/h	V cm/s	Tanks #	Q_{TOTAL} m^3/h
50	19	0.04	0.006	1	0.04
100	23	0.04	0.006	2	0.09
150	26	0.04	0.006	3	0.13
200	28	0.06	0.007	3	0.18
250	30	0.07	0.009	3	0.22
300	32	0.07	0.008	4	0.26
350	33	0.08	0.009	4	0.30
400	35	0.09	0.011	4	0.34
450	36	0.10	0.012	4	0.39
500	37	0.11	0.013	4	0.43
550	38	0.12	0.015	4	0.47
600	39	0.13	0.016	4	0.51
650	40	0.14	0.017	4	0.55
700	41	0.15	0.018	4	0.58
750	41	0.16	0.019	4	0.62
800	42	0.17	0.021	4	0.66
850	43	0.17	0.022	4	0.70
900	44	0.18	0.023	4	0.74
950	44	0.19	0.024	4	0.77
1000	45	0.20	0.025	4	0.81

Water velocity was also estimated within the culture tank. Procedures to calculate water velocity are the same shown above for water flow requirements based on oxygen consumption (Eq. 10.6). The results of the mass balance analysis show that flow rates for TAN (Table 10.6) are lower than the ones calculated for oxygen (Table 10.5). Therefore oxygen will be considered as the limiting factor regulating the rate of water flow in the Recirculating Pilot system.

10.5.3.- Water flow per tank

Water flow will be determined by oxygen need according to the values tabulated in Tables 10.5 and 10.6. Water flow per culture tank will vary between 1.8 and 8.0 m^3/h (Table 10.5). Water velocities within each of the sections in the culture tank will be less than 1 bl/s, which is the maximum relative swimming velocity recommended for the culture of California halibut.

In the design of raceways, another factor to be considered related to average channel velocity, in addition to the velocity established for the optimum relative swimming velocity, is the minimum cleaning velocity. Raceways can be self-cleaning if velocities along the bottom are greater than 6 cm/s (Burrows and Chenoweth, 1970). Table 10.5 showed that water velocities within each section of the culture tank are below 1 cm/s, hence this velocities will not be enough to carry particles to the effluent section. However, it has been observed that rearing California halibut at stocking densities >100% PCA helps to resuspended and move solids toward the effluent section (Chapter IX).

10.6.- SUBMERGED MOVING BED BIOFILTER

The tank to be used as a biofilter has the same dimensions as the fish tanks: 300 cm in length, 150 cm in width, and 76 cm in height (Fig.10.3). The biofilter has three interconnected chambers were the biofilter media will be enclosed. A section is also used for pumping. The biofilter water depth will be 66 cm. The Recirculating Pilot system will operate with make-up water of about 5% of system volume per day. The system volume will vary throughout the rearing time with the separation of the fish stock into the other tanks. The minimum make-up water will be 0.24 m^3/d and the maximum 0.49 m^3/d (Table 10.7).

The biofilter will be operated as a submerged moving bed filled with Kaldnes™ media (Table 10.8). The TAN to be nitrified in the biofilter was estimated by a mass balance on the entire system. The mass balance equation is:

$$\text{Consumption} = \text{Production} - (Q_M * C_{N\text{-}OUT}) * b \qquad \text{(Eq. 10.8)}$$

where, Consumption = TAN nitrification (mg/d)

Production = total nitrogen excreted as TAN and urea-N (mg/d)

Q_M = make-up water flow (m^3/d)

b = unit conversion factor (1000 L/m^3)

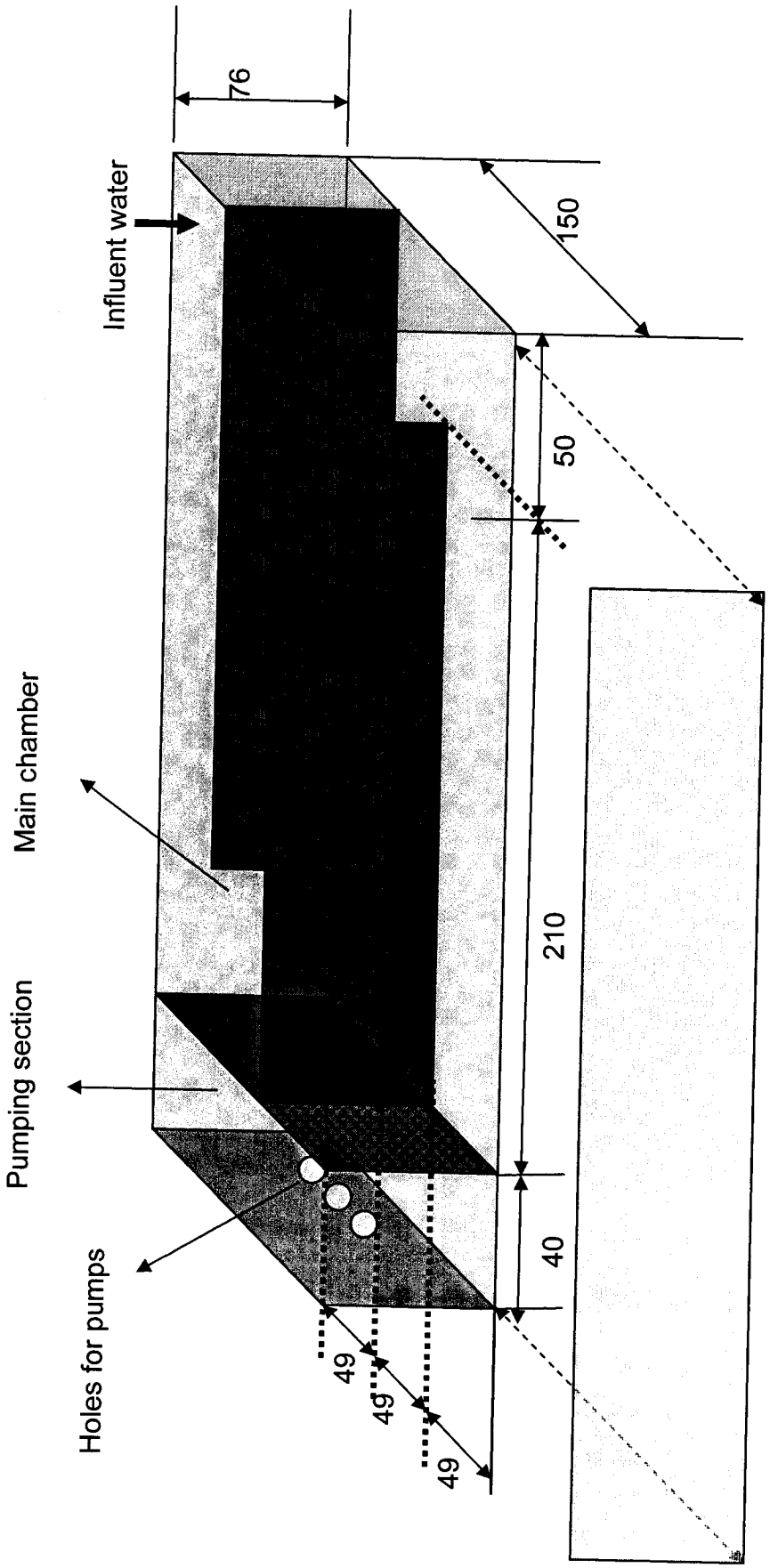

Figure 10.3.- Schematic diagram of the biofilter tank no to scale (units in cm). Biomedia will be placed within the main chamber, which has been subdivided in three channels. A screen separates the biomedia section from the pumping section. Influent water comes from the drum filter.

Table 10.7.- Total Recirculating Pilot system volume. Pipes were assumed to have 1% of the total volume content. Make-up water is assumed to be 5% of the total system volume.

Fish tanks #	TANKS m^3	SWIRLS m^3	Biofilter m^3	Pipes m^3	TOTAL m^3	Make-up m^3/d
1	1.35	0.34	2.97	0.05	4.70	0.24
2	2.70	0.67	2.97	0.06	6.40	0.32
3	4.05	1.01	2.97	0.08	8.11	0.41
4	5.40	1.34	2.97	0.09	9.81	0.49

Table 10.8.- Technical specifications for Kaldnes™ filter media (http://www.W-M-T.com). SNR stands for media surface nitrification rate and VNR for media volumetric nitrification rate.

Specifications	
Material	Polyethylene
Specific surface area	500 m^2/m^3
Maximum fill	up to 70%
Bulk density	152 kg/m^3
Number of units per m^3	1,029,000
Surface area per unit	4.86 cm^2
Percentage of void space	93%
Color	natural white
SNR (mg/m^2 d)	100[a] to 300[b]
VNR (g/m^3 d)	51[a] to 150[b]
Material density	0.96 g/cm^3 [c]

a) data from Chapter IX (Table 9.8)
b) data from PRAqua Technologies Ltd. (Sean Wilton, personal communication)
c) Helm et al., 1994; Rusten et al., 1995

Note: Data measured in the present research (Chapter IX) were used for sizing the submerged moving bed biofilter.

Assumptions include a steady state condition, 0 mg TAN/L in the make-up water, and 1 mg TAN/L ($C_{N\text{-}OUT}$) overflowing in the pumping section of the biofilter tank (overflow not shown in Fig. 10.3). Total nitrogen (TN) excreted by the fish includes both the TAN and the urea-N excretion (Table 10.9).

The maximum volume of Kaldnes™ media required will be 1.3 m^3 if it is assumed that media nitrification rate (SNR) is 100 mg TAN/m^2 d (Table 10.8). The biomedia will use up to 51% of the main biofilter chamber volume (Table 10.9).

Oxygen needed for nitrification and alkalinity consumed by nitrification were calculated for the TAN to be nitrified ("Nitrification" column in Table 10.9) and presented in Table 10.10. The overall nitrification reaction requires 4.57 g of O_2 and about 0.14 eq of alkalinity for the complete oxidation of 1 g of TAN (Malone and DeLosReyes, 1997; Loyless and Malone, 1997; Timmons et al., 2001). System alkalinity will be kept at a minimum of 2.4 meq/L (Chapter 9, Table 9.6). A maximum consumption of 296 g O_2/d and 9.7 eq/d will be needed to nitrify 64.8 g TAN/d (Table 10.10).

The biofilter will have an extensive air diffuser system, hence it was assumed that the biofilter effluent would be at saturation (~7.48 mg O_2/L; see section 10.5.1). Additional assumptions included a steady state condition and a dissolved oxygen concentration of 5 mg/L entering the biofilter (see section 10.5.1). Hence a mass balance for oxygen in the biofilter is:

Table 10.9.- Determination of media surface area (MSA) and media volume (MV) for Kaldnes™ required for the biofilter. TAN and urea-N excretion per day are shown as total nitrogen (TN). Biofilter main chamber volume (BFV) is 2.57 m^3.

Biomass g	Total fish #	Total biomass kg	Tanks #	N-feed g/d	Make-up m^3/d	TN mg/d	Nitrification mg/d	MSA m^2	MV m^3	MV / BFV %
50	682	34	1	23	0.24	3590	3354	34	0.1	4
100	679	68	2	47	0.32	7143	6823	68	0.1	4
150	675	101	3	70	0.41	10661	10256	103	0.2	8
200	672	134	3	92	0.41	14144	13738	137	0.3	12
250	668	167	3	115	0.41	17591	17186	172	0.3	12
300	665	200	4	137	0.49	21004	20513	205	0.4	16
350	662	232	4	159	0.49	24382	23892	239	0.5	19
400	658	263	4	181	0.49	27726	27235	272	0.5	19
450	655	295	4	203	0.49	31036	30545	305	0.6	23
500	652	326	4	224	0.49	34312	33821	338	0.7	27
550	649	357	4	245	0.49	37554	37064	371	0.7	27
600	645	387	4	266	0.49	40763	40273	403	0.8	31
650	642	417	4	287	0.49	43939	43449	434	0.9	35
700	639	447	4	308	0.49	47083	46592	466	0.9	35
750	636	477	4	328	0.49	50194	49703	497	1.0	39
800	633	506	4	348	0.49	53272	52782	528	1.1	43
850	629	535	4	368	0.49	56319	55828	558	1.1	43
900	626	564	4	388	0.49	59333	58843	588	1.2	47
950	623	592	4	407	0.49	62317	61826	618	1.2	47
1000	620	620	4	427	0.49	65268	64778	648	1.3	51

MSA = Nitrification/SNR
SNR = media surface nitrification rate = 100 mg/m^2 d (Table 10.8)
MV = MSA / SSA
SSA = media specific surface area = 500 m^2/m^3 (Table 10.8)

Table 10.10.- Oxygen and alkalinity daily requirements for TAN nitrification.

Fish Size g	Total fish #	TAN Nitrification g/d	Oxygen for nitrification g/d	Alkalinity for Nitrification eq/d
50	682	3.4	15	0.5
100	679	6.8	31	1.0
150	675	10.3	47	1.4
200	672	13.8	63	1.9
250	668	17.2	79	2.4
300	665	20.5	94	2.9
350	662	23.9	109	3.3
400	658	27.2	124	3.8
450	655	30.6	140	4.3
500	652	33.8	155	4.7
550	649	37.1	169	5.2
600	645	40.3	184	5.6
650	642	43.5	199	6.1
700	639	46.6	213	6.5
750	636	49.7	227	7.0
800	633	52.8	241	7.4
850	629	55.8	255	7.8
900	626	58.8	269	8.2
950	623	61.8	283	8.7
1000	620	64.8	296	9.1

TAN nitrification was estimated in Table 10.9.

Oxygen for nitrification was estimated from 4.57 g O_2/g TAN.

Alkalinity used by nitrification was estimated based on 0.14 eq/g TAN.

$$\text{Production} = \text{Consumption} + Q_{TOTAL} (C_{OUT} - C_{IN}) * n \qquad \text{(Eq. 10.9)}$$

where, Production = oxygen required (g/d)

Consumption = oxygen for nitrification (g/d) (Table 10.10)

Q_{TOTAL} = system water flow (m^3/d) (Table 10.11)

C_{IN} = dissolved oxygen entering (mg/L)

C_{OUT} = dissolved oxygen leaving (mg/L)

n = factor to convert units (24 L g h / m^3 mg d)

From this mass balance it was determined that a maximum of 2200 g/d of oxygen has to be produced within the biofilter when the water flow entering the biofilter is 32 m^3/h (Table 10.11). This 2200 g/d of oxygen includes the oxygen required for nitrification and the oxygen required to reach a saturated biofilter effluent.

10.7.- AERATION AND/OR OXYGENATION

A continuous supply of adequate amounts of dissolved oxygen to fish in the culture tanks and bacteria in the biofilter are essential for a recirculating system. As indicated above the submerged moving bed biofilter requires a considerable amount of air for mixing, which provides the oxygen required for nitrification. Oxygenation of the water for the fish will be based on the use of pure oxygen.

Table 10.11.- Oxygen to be produced within the biofilter tank. The biofilter effluent will be at 7.48 mg O_2/L. Total flow is from Table 10.5 and oxygen for nitrification is from Table 10.10.

Fish Size g	Tanks #	Total Flow m^3/h	Oxygen for nitrification g/d	Oxygen to be produced g/d
50	1	1.8	15	120
100	2	3.5	31	240
150	3	5.2	47	358
200	3	6.9	63	475
250	3	8.6	79	592
300	4	10.3	94	706
350	4	12.0	109	820
400	4	13.6	124	933
450	4	15.2	140	1045
500	4	16.8	155	1156
550	4	18.4	169	1265
600	4	20.0	184	1373
650	4	21.5	199	1480
700	4	23.1	213	1586
750	4	24.6	227	1691
800	4	26.1	241	1795
850	4	27.6	255	1898
900	4	29.1	269	2000
950	4	30.5	283	2100
1000	4	32.0	296	2200

The oxygen unit will be sized to meet the maximum oxygen requirements. Table 10.12 shows the dissolved oxygen requirement per day for the fish and the net oxygen production by aeration within the biofilter unit. The maximum oxygen requirement is ~2870 g O_2/d (Table 10.12). A mass balance to the entire Recirculating Pilot system was performed to determine the amount of oxygen required per day. The assumptions included steady state conditions, influent make-up water saturated at 7.48 mg O_2/L, and effluent water at 5 mg O_2/L. The maximum oxygen requirements calculated for the Recirculating Pilot system are 1.4 L/min assuming 100% transfer efficiency (Table 10.13).

10.7.1.- Packed column and oxygen generator

One of the most effective process configurations for gas transfer is a countercurrent packed column. A packed column has a supported mass of packing media through which water trickles down while oxygen gas is blown from the bottom. There are three design parameters required for the design of absorption columns: column cross section, column height, and gas volumetric: liquid flow ratio (G:L). The cross section is a function of the water flow rate through the section. The G:L ratio for pure oxygen systems addition should be between 0.3 and 5% (Timmons and Losordo, 1994; Lawson, 1995). And the column depth can be determined from (Watten, 1990; Watten et al., 1991):

$$\ln\left(\frac{C^* - C_{in}}{C^* - C_{out}}\right) = KZ + nK_d \qquad \text{(Eq. 10.10)}$$

Table 10.12.- Dissolved oxygen requirements for the Recirculating Pilot system.

Fish Size g	Tanks #	Oxygen for fish[a] g/d	Net oxygen from biofilter[b] g/d	Required oxygen[c] g/d
50	1	263	105	158
100	2	523	208	314
150	3	780	311	469
200	3	1035	413	622
250	3	1287	513	774
300	4	1536	613	924
350	4	1784	711	1072
400	4	2028	809	1219
450	4	2270	905	1365
500	4	2510	1001	1509
550	4	2747	1096	1652
600	4	2982	1189	1793
650	4	3214	1282	1932
700	4	3444	1374	2071
750	4	3672	1464	2207
800	4	3897	1554	2343
850	4	4120	1643	2477
900	4	4340	1731	2609
950	4	4558	1818	2740
1000	4	4774	1904	2870

a) Oxygen for fish was estimated from oxygen data per tank presented in Table 10.4 times the number of tanks used.

b) The "Net oxygen from biofilter" column is the oxygen to be produced in the biofilter beyond that needed for nitrification (Table 10.11).

c) "Required oxygen" column is obtained from the "oxygen for fish" minus "net oxygen from biofilter".

Table 10.13.- Oxygen to be generated in the Recirculating Pilot system by an oxygen generator assuming 100% transfer efficiency. At standard temperature and pressure conditions, 32 g O_2 are equivalent to 22.4 L of volume.

Fish Size g	Tanks #	Required oxygen[a] g/d	Make-up water[b] m^3/d	Oxygen Generated g/d	Oxygen generated L/min
50	1	158	0.24	157	0.1
100	2	314	0.32	313	0.2
150	3	469	0.41	468	0.2
200	3	622	0.41	621	0.3
250	3	774	0.41	773	0.4
300	4	924	0.49	922	0.4
350	4	1072	0.49	1071	0.5
400	4	1219	0.49	1218	0.6
450	4	1365	0.49	1364	0.7
500	4	1509	0.49	1508	0.7
550	4	1652	0.49	1650	0.8
600	4	1793	0.49	1791	0.9
650	4	1932	0.49	1931	0.9
700	4	2071	0.49	2069	1.0
750	4	2207	0.49	2206	1.1
800	4	2343	0.49	2342	1.1
850	4	2477	0.49	2475	1.2
900	4	2609	0.49	2608	1.3
950	4	2740	0.49	2739	1.3
1000	4	2870	0.49	2869	1.4

a) "Required oxygen" data from Table 10.12

b) "Make-up water" data from Table 10.7

where, C^* = saturation gas concentration in the column (mg/L)

C_{in} = gas concentration at the inlet (mg/L)

C_{out} = gas concentration at the outlet (mg/L)

K = mass transfer coefficient for packing media (1/m)

Z = depth of packing media (m)

n = number of distribution plates within the column (dimensionless)

K_d = distribution plate mass transfer coefficient (dimensionless)

The term nK_d is between 0.3 and 0.4 and represents the expected gas transfer across the distribution plate placed over the packing media (Watten et al., 1991; Lawson, 1995). Therefore the column depth can be determined from (Lawson, 1995):

$$Z = \frac{\ln\left(\frac{C^* - C_{in}}{C^* - C_{out}}\right) - 0.4}{K} \qquad \text{(Eq. 10.11)}$$

The saturation oxygen concentration in the column can be calculated from:

$$C^* = C_s \frac{P_{column}}{P_{atm}} \qquad \text{(Eq. 10.12)}$$

where, C_s = oxygen saturation at field conditions (mg/L)

P_{column} = pure oxygen mole fraction in the column (90%)

P_{atm} = atmospheric oxygen mole fraction (20.9%)

Dissolved oxygen concentration is ~7.48 mg/L (at salinity 30 g/L and temperature 21 °C) in the water entering the column, and 11.22 mg/L is required in the water leaving the column (see section 10.5.1). The oxygen mole fraction in the column is assumed to be equal to that of the gas produced by a VSA oxygen generator (90±2%) (http://www.airproducts.co.uk/aquaculture/VSA_specifications.htm).

Under the present operation conditions Eq. 10.11 gave a negative Z value for the packed column to be designed. This is due to the small oxygen concentration increase from influent to effluent and the high transfer expected across the distribution plate (nK_d = 0.4). Hence packing media height within the column will be arbitrarily chosen to be 0.5 m. The fish tanks have a water depth of 0.3 m. Packing media will be located above the tank water level in columns resting on the bottom of the tanks. Water entrance to the packed column will be at 0.2 m above the top level of the packing media. The total column height will be 1 m and the diameter will be 15 cm (Table 10.14; Fig. 10.4).

The gas transfer efficiency for packed columns depends upon the column packing height, the hydraulic loading, and the characteristic of the packing material. Packet column absorption efficiency reported is between 40 and 80% (Timmons and Losordo, 1994; Watten et al., 1991; Lawson, 1995). For the

purposes of the present design an efficiency of 50% will be chosen and two columns will be installed in each tank (Fig. 10.4). Therefore the volume of oxygen required can be estimated from the oxygen requirements shown in Table 10.13 (Table 10.14):

$$Q_{gr} = \frac{Q_G}{\eta} \qquad \text{(Eq. 10.13)}$$

where, Q_{gr} = gas flow required in the column (L/min)

Q_G = oxygen generated (L/min) (Table 10.13)

η = gas transfer efficiency in the column (50%)

The maximum packed column water flow will be 4.0 m^3/h and a maximum of 0.35 L/min of gas flow will be required for an efficiency of oxygen transfer of 50% (Table 10.14). Therefore a total of 2.8 L/min of oxygen are required for the eight packed columns to be installed within the rearing tanks (Fig. 10.4). A VSA oxygen generator model A-005L will be chosen to provide the amount of gas required (http://www.airproducts.co.uk/aquaculture/pdf/a_005l.pdf), which delivers up to 5 L/min of gas containing 90% oxygen. Technical characteristics of model A-005L are presented in Table 10.15.

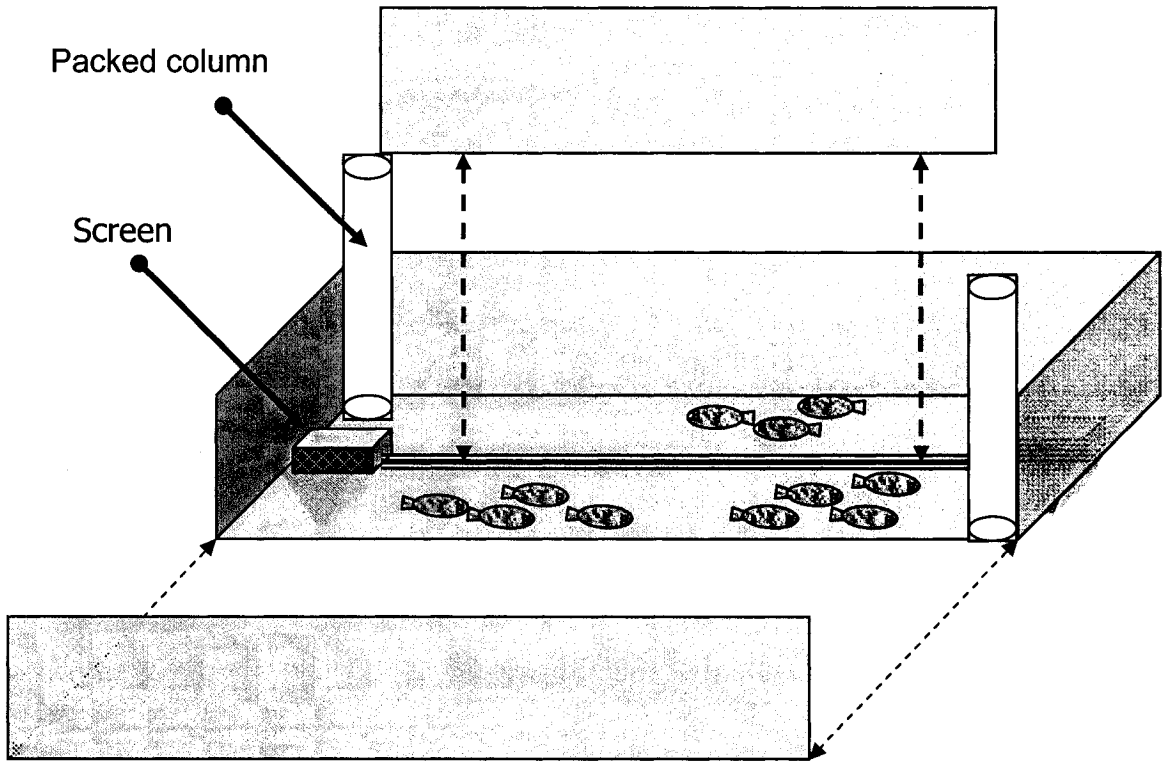

Figure 10.4.- Fish culture tank with the packed columns. Packed columns are located in opposite corner within the tank.

Table 10.14.- Single packed column dimensions and operation characteristics. Two columns operate in each tank.

Water flow per column m^3/h	Gas flow per column L/min	G:L ratio	Column cross area m^2	Column diameter cm
0.9	0.08	0.005	0.004	7
0.9	0.08	0.005	0.004	7
0.9	0.08	0.005	0.004	7
1.2	0.10	0.005	0.005	8
1.4	0.13	0.005	0.007	9
1.3	0.11	0.005	0.006	9
1.5	0.13	0.005	0.007	9
1.7	0.15	0.005	0.008	10
1.9	0.17	0.005	0.009	10
2.1	0.18	0.005	0.010	11
2.3	0.20	0.005	0.010	12
2.5	0.22	0.005	0.011	12
2.7	0.23	0.005	0.012	12
2.9	0.25	0.005	0.013	13
3.1	0.27	0.005	0.014	13
3.3	0.28	0.005	0.015	14
3.5	0.30	0.005	0.016	14
3.6	0.32	0.005	0.017	15
3.8	0.33	0.005	0.017	15
4.0	0.35	0.005	0.018	15

Media size 8.89 cm; K at 20 °C is 1.05; Loading is 220 m^3/m^2 h (Lawson, 1995).

Packed column oxygen transference efficiency was assumed to be 50%.

Table 10.15.- Specifications of VSA oxygen generator model A-005L (http://www.airproducts.co.uk/aquaculture/VSA_specifications.htm).

Specifications	Range
Flow rate (L/min)	5
Oxygen content (%)	90 ± 2
Delivery pressure (bar)	0.41 to 0.34
Power consumption (kWh)	0.25

10.8.- SOLIDS REMOVAL

Suspended solids can cause a degradation in water quality (Chen et al., 1993a, 1993b) that will create a variety of problems including, physiological stress on the fish (Poxton and Allouse, 1982; Wedemeyer, 1996). Solids can decrease dissolved oxygen levels as they decay (Cripps and Bergheim, 2000; Sumagaysay-Chavoso and San Diego-McGlone, 2003). They can also leach nutrients and toxic substances such as hydrogen sulfide (Wyban and Sweeny, 1989). Turbidity, due to suspended solids, may also interfere with sight feeding fish species, resulting in poor feed uptake (Timmons et al., 2001).

In the design of clarifiers a critical particle terminal velocity, V_{sc}, is used as the design overflow settling velocity (Mihelcic, 1999). All particles within the clarifier that have a terminal velocity V_s equal to or greater than V_{sc} will settle. The V_{sc} is equal to the overflow rate (OFR), defined as the volumetric flow rate, Q, entering the basin, divided by its surface area, A (Wong and Piedrahita, 2000; Lin, 2001).

Data obtained from settling velocity tests can be used to construct a cumulative settling velocity distribution curve showing the proportion of material by mass, with settling velocities less than a given value. Settling velocity distribution for particles settled within raceways where California halibut have been reared are shown in Fig. 10.5 (see Chapter VIII). Settling velocity distributions can be used to evaluate the solids removal effectiveness of a given clarifier.

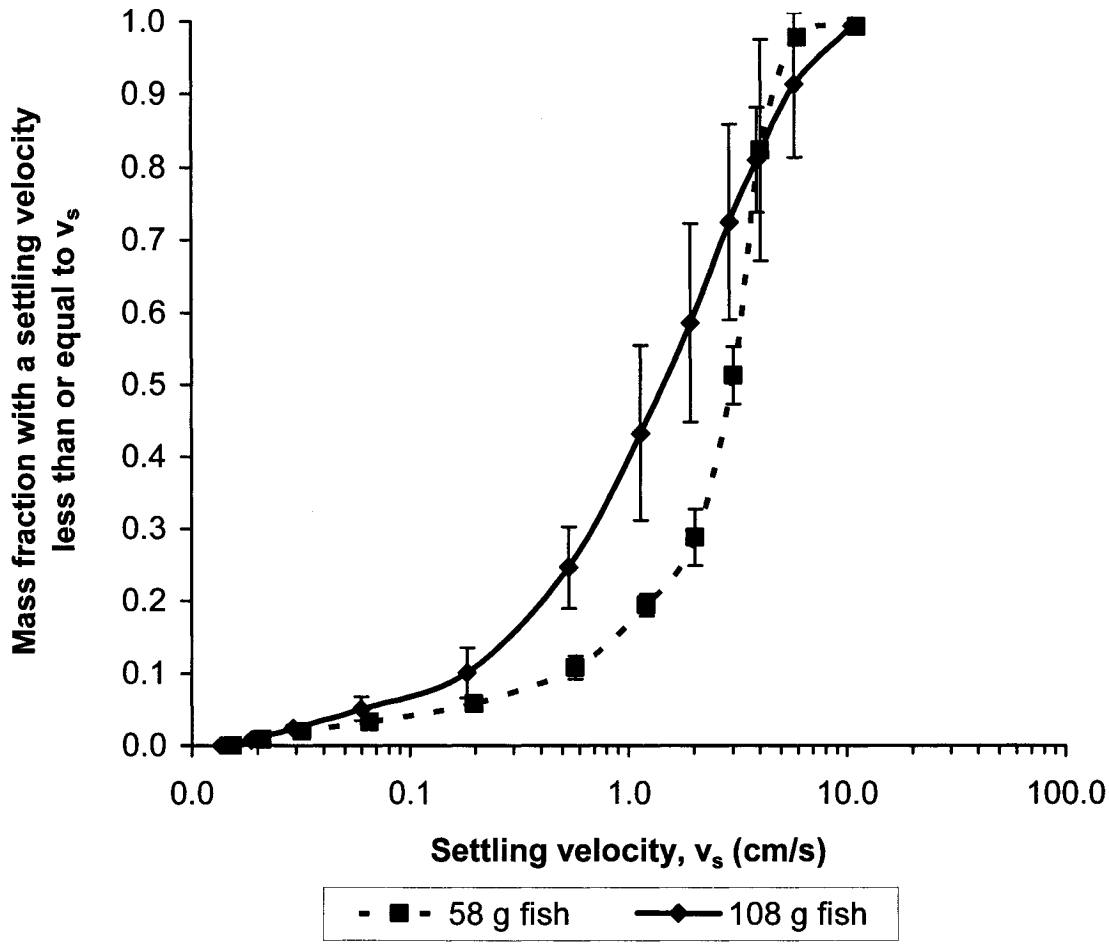

Figure 10.5.- Settling velocity curves for particles settled within the raceways used for the culture of California halibut. Two curves are shown, one for fish weighing 58 g and another for fish weighing 108 g. Fish were fed exclusively with EWOS ^{TM} 3 mm (See Chapter VIII).

10.8.1.- Swirl separator

The swirl separator is a cylindrical cone-shaped tank that forces the water to rotate about its axis to enhance particle settling (Timmons et al., 2001). Loading rates for swirl separators are approximately four times greater than the recommended loading rates for conventional settling basins (Paul et al., 1991; Timmons et al., 2001). Recommended surface loading rates are between 0.19 and 0.33 cm/s, and hydraulic retention times should not be less than 30 s (Timmons et al., 2001). Swirl separators are effective in removing up to 87% of solids greater than 77 μm (Scott and Allard, 1984). Removal efficiencies for the swirl separators varied from 10% to 90% and it was found to increase with increasing inlet concentration with large particles (>100 μm) being effectively removed (Brooks and Couturier, 2000). It has been stated with respect to sewer overflows that the practical lower limit of swirl separation is a particle with a settling velocity of 0.10 to 0.14 cm/s (CASQA, 2004).

A swirl separator will be installed at the Recirculating Pilot facility at Bodega Marine Laboratory, as the first solids removal device. The swirl separator to be used has a diameter of 76.2 cm and an inlet pipe of 10 cm diameter with a ~0.34 m^3 working volume (Fig. 10.6 and Fig. 10.7).

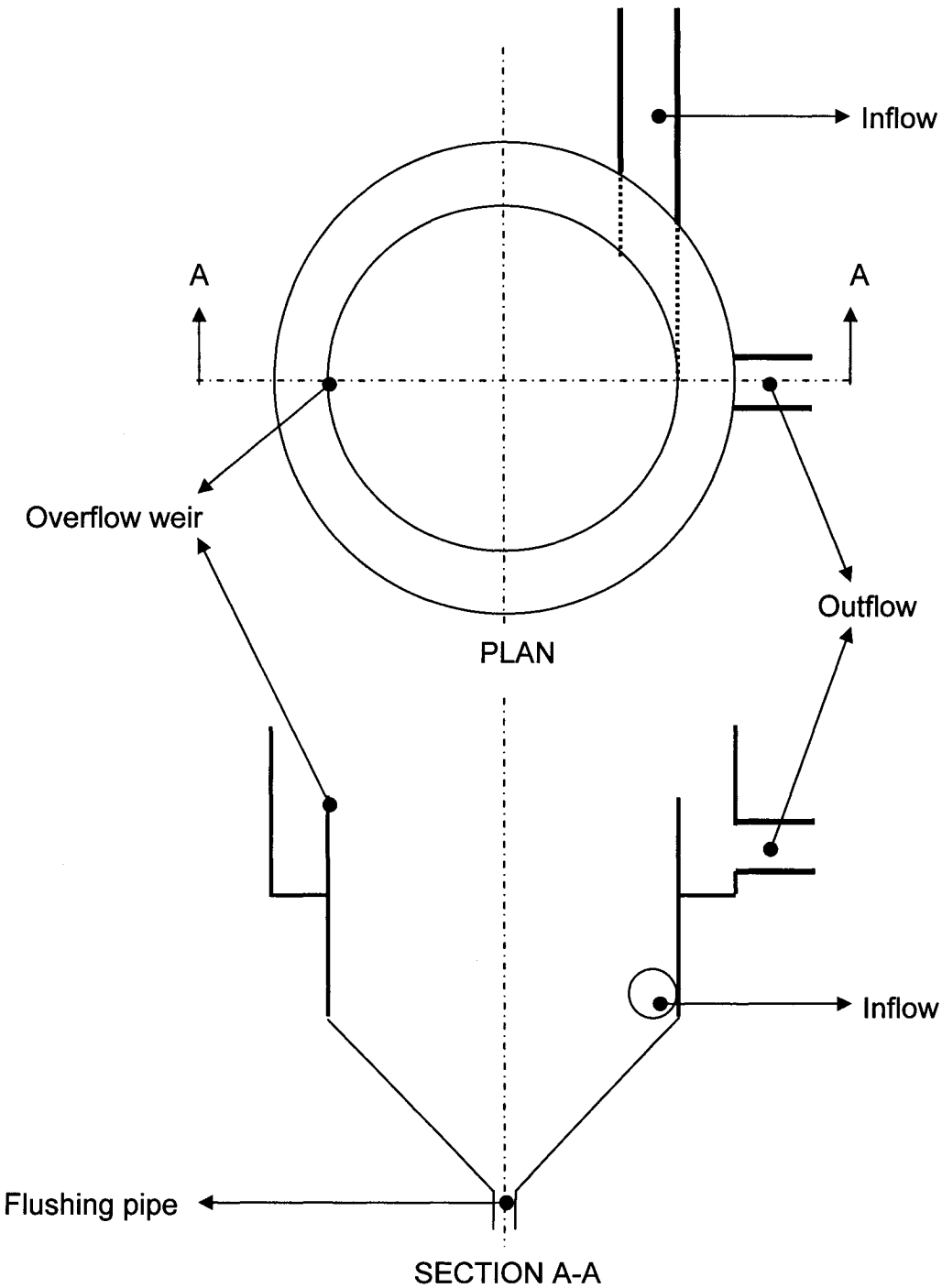

Figure 10.6.- Schematic diagram of a swirl separator to be used at the Recirculating Pilot facility at Bodega Marine Laboratory. Not to scale. Swirl separator volume is 0.34 m^3.

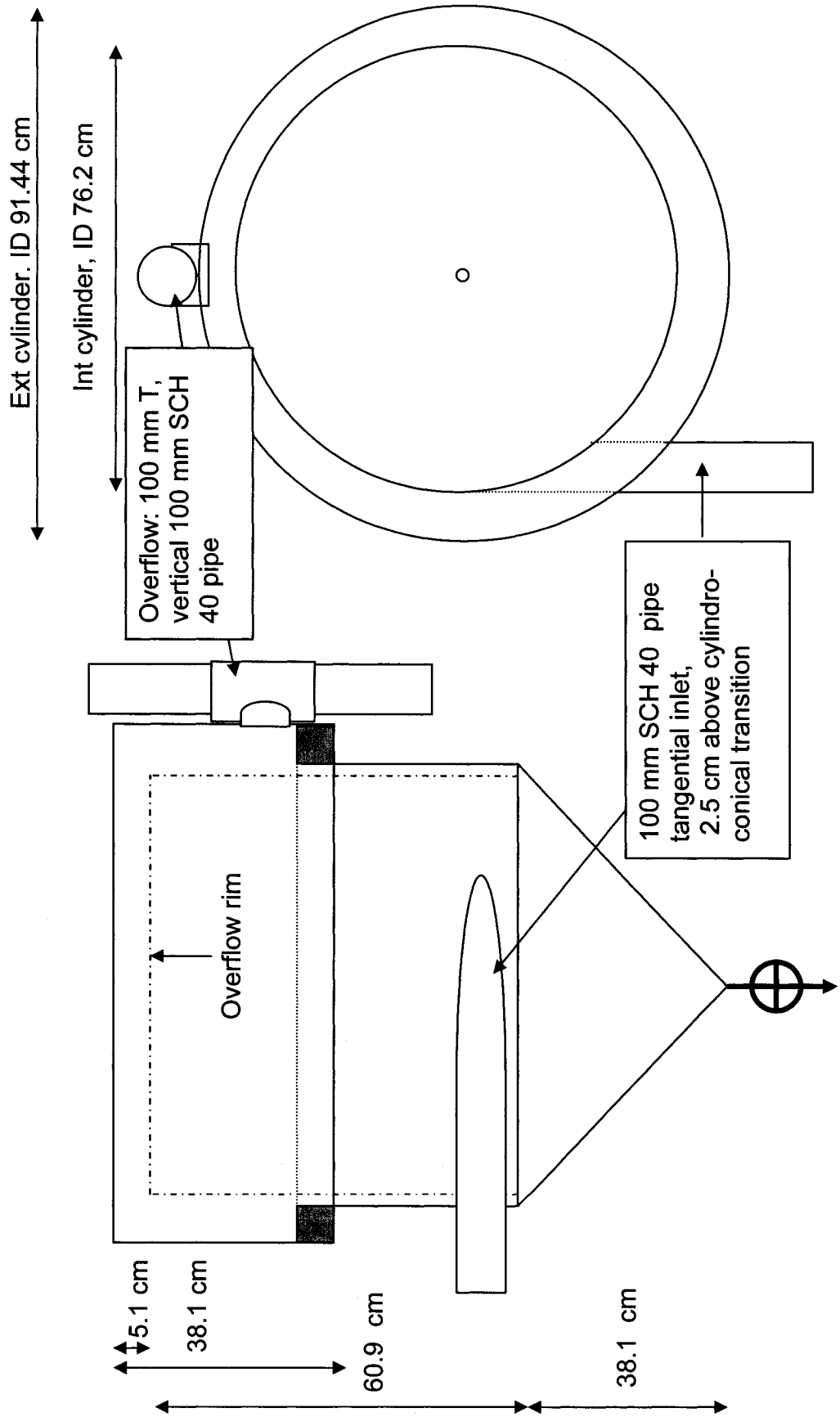

Figure 10.7.- Schematic diagram of swirl separator to be used in the Recirculating Pilot system. Not to scale.

It has been reported through computer models analysis that the dominant mode of particle removal in swirl separators is gravitational and that a tangential inlet does little to improve removal efficiencies though increasing centrifugal forces (Brooks and Couturier, 2000). Consequently the calculation of solids removed in the present swirl separator can be approached with the equation for the theoretical overall removal efficiency (η) of a clarifier (Lin, 2001; Chapter VIII, item 8.1.1.4):

$$\eta = (1 - Fo) + \frac{1}{Vsc} \int_{0}^{Fo} VsdF \qquad \text{(Eq. 10.14)}$$

Swirl separator hydraulic retention time (HRT), OFR, and theoretical removal for possible operational conditions at the recirculating pilot facility are shown in Table 10.16. Between 95 and 86% of the solids generated within the culture tanks are expected to be removed within the swirl separator for an OFR range between 0.11 and 0.49 cm/s (Table 10.16). The performance of the swirl separator to be used is within the ranges reported from literature (Scott and Allard, 1984; Brooks and Couturier, 2000; CASQA, 2004).

In addition, it has been reported for salmonids that 1 kg of feed produces about 0.3 kg of fecal solids (Smith et al., 1980; Beveridge et al., 1991; Bergheim and Brinker, 2003; Patterson and Watts, 2003). If this relationship were true for California halibut, then between 97 and 400 g/d of solids should be expected to be settled within the swirl separators (Table 10.17). These assumptions will be studied during the operation of the Recirculating Pilot facility.

Table 10.16.- Swirl separator hydraulic retention time (HRT) and overflow rate (OFR) determined under possible operational conditions at the Recirculating Pilot facility. Swirl separator working volume is ~0.34 m^3 and overflow rim diameter is 76.2 cm.

Fish size g	Flow per tank[1] m^3/h	Swirl HRT min	Swirl OFR cm/s	Particle removal %
50	1.8	11.5	0.11	95
100	1.8	11.5	0.11	95
150	1.7	11.6	0.11	95
200	2.3	8.7	0.14	94
250	2.9	7.0	0.18	93
300	2.6	7.8	0.16	93
350	3.0	6.7	0.18	93
400	3.4	5.9	0.21	92
450	3.8	5.3	0.23	91
500	4.2	4.8	0.26	90
550	4.6	4.4	0.28	89
600	5.0	4.0	0.30	89
650	5.4	3.7	0.33	88
700	5.8	3.5	0.35	88
750	6.2	3.3	0.37	87
800	6.5	3.1	0.40	87
850	6.9	2.9	0.42	87
900	7.3	2.8	0.44	86
950	7.6	2.6	0.47	86
1000	8.0	2.5	0.49	86

1) "Flow per tank" data from Table 10.5.

Table 10.17.- Expected mass of particles to be settled daily in each swirl separator. Operating OFRs and particle removal percentages were given in Table 10.16. California halibut will be fed daily at 1% BW and 0.3 kg of solids are assumed to be produced per kg of feed.

Fish size g	Total fish #	Tanks #	Fish per tank	Biomass kg/tank	Feed g/tank	Solids g/tank d	Swirl g/d
50	682	1	682	34	341	102	97
100	679	2	339	34	339	102	97
150	675	3	225	34	338	101	96
200	672	3	224	45	448	134	126
250	668	3	223	56	557	167	155
300	665	4	166	50	499	150	139
350	662	4	165	58	579	174	162
400	658	4	165	66	658	198	182
450	655	4	164	74	737	221	201
500	652	4	163	81	815	244	220
550	649	4	162	89	892	268	238
600	645	4	161	97	968	290	258
650	642	4	161	104	1044	313	275
700	639	4	160	112	1118	335	295
750	636	4	159	119	1192	358	311
800	633	4	158	127	1265	380	330
850	629	4	157	134	1338	401	349
900	626	4	157	141	1409	423	364
950	623	4	156	148	1480	444	382
1000	620	4	155	155	1550	465	400

10.8.2.- Microscreen filtration

Knowledge of the size distribution of particles can be used in determining the size of the screening device to be used for their removal (Cripps, 1993; Lawson, 1995). Libey (1993) reported that a drum filter with a 40 μm screen mesh size could remove 40% of the suspended solids. However, drum filters with a screen pore size of 60 to 100 μm have been found to be most cost-effective for aquacultural applications, since below that range an increase in filtration effort no longer improved solids removal (Kelly et al., 1997; Cripps and Bergheim, 2000; Vinci et al., 2001).

In the absence of information on the size of particles, Stoke's law can be used to estimate particle size from settling velocity data (Fig. 10.5). Stoke's law defines the fundamental equation used to calculate the terminal settling velocities of particles less than 70 μm (Loch, 2001) in both air and water at low Reynold's number (R<2) (Lin, 2001):

$$v_s = \frac{g(\rho_p - \rho_f)}{18\mu} D_p^2 \qquad \text{(Eq. 10.15)}$$

$$R = \frac{\rho_f v D}{\mu} \qquad \text{(Eq. 10.16)}$$

where v_s = particle settling velocity (m/s)

g = acceleration due to gravity (9.81 m/s^2)

ρ_p = particle density (kg/m^3)

ρ_f = water density (kg/m^3)

D_p = particle diameter (m)

μ = fluid viscosity (kg / m s)

R = Reynold's number

Attempts have been made in aquaculture to determine the densities of solids of feed origin. Chen et al. (1993b) reported an overall mean density of 1190 kg/m^3 (specific gravity of 1.19). Patterson et al. (2003), studying the solids from more than 10 fish farms, stated that the heaviest particles have a mean density of 1153 kg/m^3 and the lightest a mean of 1050 kg/m^3. Assuming that particles settled within the California halibut raceways have a density of 1150 kg/m^3, particle size corresponding to a given settling velocity was estimated using Stoke's law. According to Fig. 10.8 the minimum particle size to be removed by the swirl separator will be between 120 and 300 µm for the OFR operational range of 0.11 to 0.49 cm/s (Table 10.16).

A drum filter (PRAqua Technologies Ltd model RFM2014) with a 60 µm screen was chosen for the Recirculating Pilot system. White and Townsend (1996) evaluated PRAqua drum filters with screen sizes of 9, 16, 30, and 60 µm. During this evaluation, water containing particles up to 300 µm was used. The 60 µm screen size removed all of the particles 32.84 µm and larger. The estimated minimum particle size settled within the raceways rearing California halibut larger than 50 g was ~43 µm (Fig. 10.8). Therefore, the drum filter is expected to remove all settleable solids that did not settle within the swirl separator.

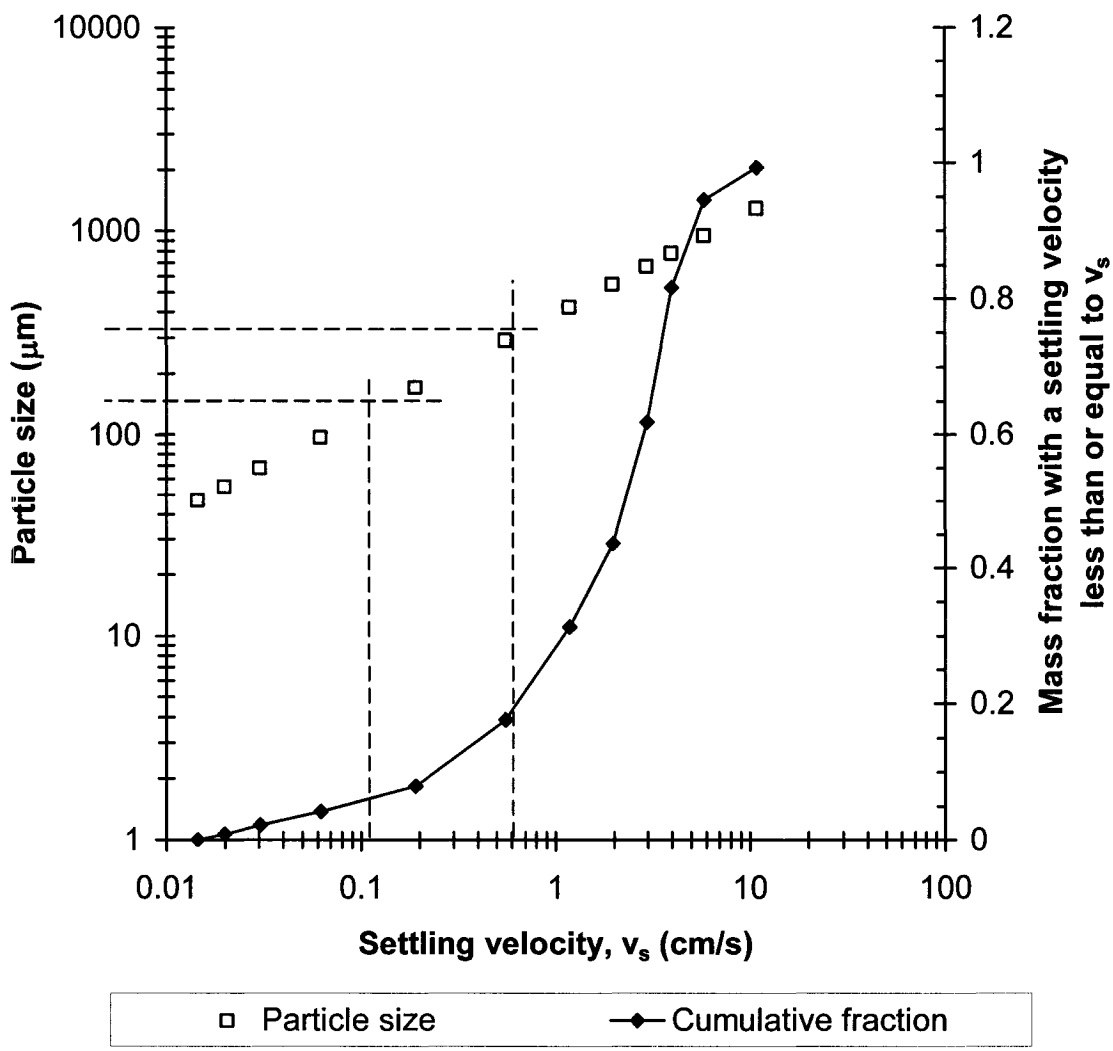

Figure 10.8.- Particle size and settling velocity distribution for solids settled within raceways rearing California halibut larger than 50 g. Data plotted in this graph are averages of those shown in Fig. 10.5. Dashed lines show the particle size range to be removed by the swirl separator, depending on operational OFR (Table 10.16).

10.9.- HYDRAULIC DESIGN AND PUMP SELECTION

This section deals with the elevation of system components and the selection of pipe diameters and pumps. All calculations are made for peak flow rates.

10.9.1.- Fish tank and water treatment devices elevations

In the Recirculating Pilot system the highest point in terms of water flow will be the fish culture tank and the lowest point will be the biofilter. The swirl separator was chosen as the reference elevation for rearing tanks, drum filter, and biofilter elevation water levels (Fig. 10.9).

Bernoulli's equation was used to determine the elevations for the fish culture tanks, the drum filter, and the biofilter (Chadwick and Morfett, 1998):

$$Z_1 + \frac{P_1}{\rho g} + \frac{V_1^2}{2g} = Z_2 + \frac{P_2}{\rho g} + \frac{V_2^2}{2g} + h_f \qquad \text{(Eq. 10.17)}$$

where, Z = potential or elevation (m)

$P/\rho g$ = pressure head (m)

$V^2/2g$ = kinetic or velocity head (m)

h_f = pipe friction head loss (m)

P = pressure (N/m^2)

ρ = fluid density (kg/m^3)

g = gravitational acceleration (9.81 m/s^2)

V = fluid velocity (m/s)

Subscripts 1 and 2 refer to two points along any streamline

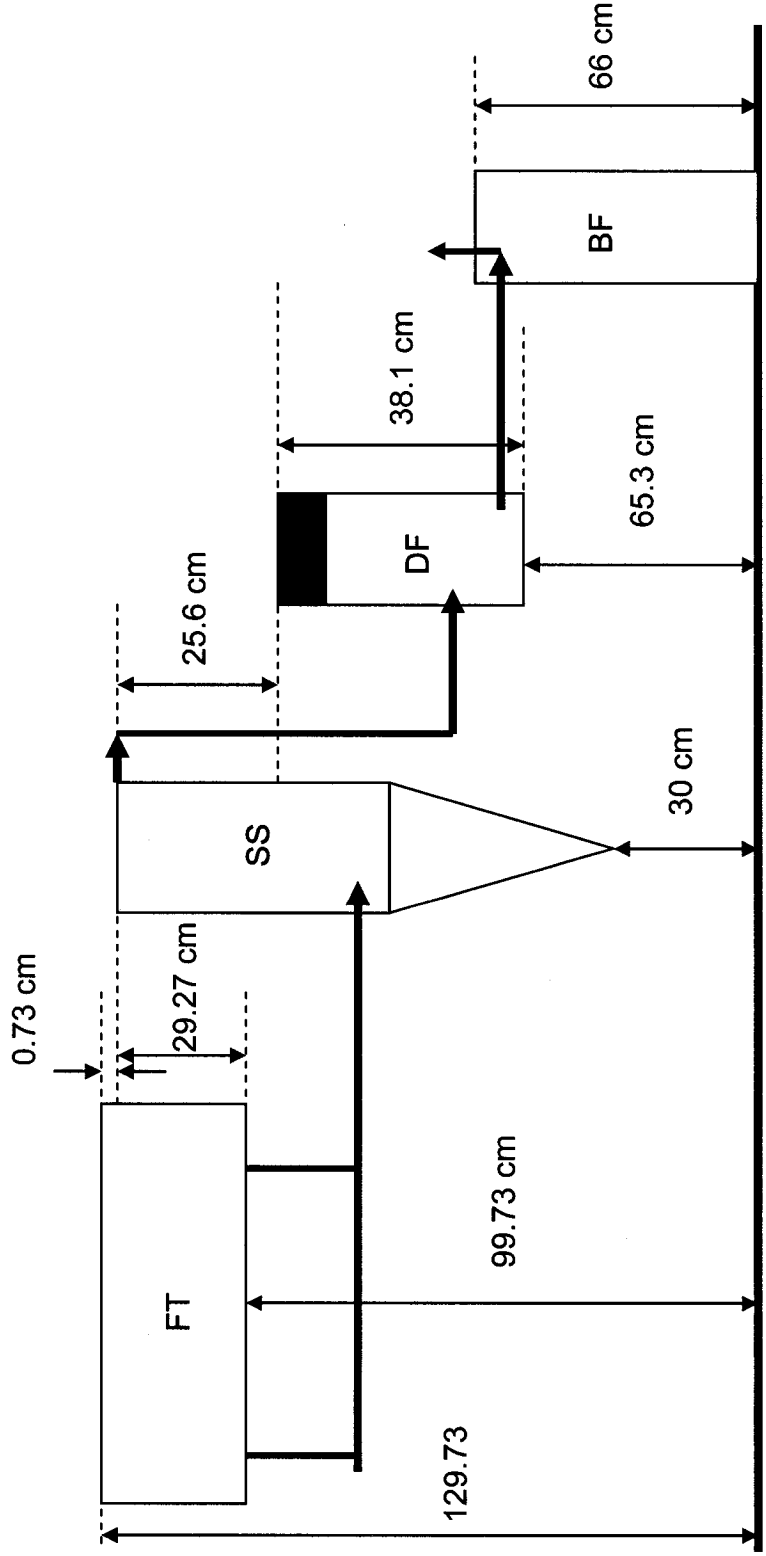

Figure 10.9.- Elevations of the units within the Recirculating Pilot system. Only free surfaces are shown in this schematic. Drawing not to scale. FT: fish tank; SS: swirl separator; DF: drum filter; BF: biofilter. The drum filter is analyzed for the maximum operational depth: black box stand for 7.6 cm of extra depth before backwash is activated, and the normal operation depth is 30.5 cm (PRAqua Technologies Ltd). SS is 30 cm off the floor for easier access to the flushing valve.

Pipe friction head losses were estimated with the Darcy-Weisbach equation (Chadwick and Morfett, 1998):

$$h_f = f \frac{L}{D} \frac{V^2}{2g} \qquad \text{(Eq. 10.18)}$$

where, f = friction factor (dimensionless)

L = total straight length of pipe (m)

D = inside pipe diameter (m)

The friction factor was estimated using the Blasius equation, which is valid for Reynolds (Re) numbers between 4000 and 100000 (Larock et al., 2000):

$$f = \frac{0.316}{R_e^{0.25}} \qquad \text{(Eq. 10.19)}$$

$$R_e = \frac{VD\rho}{\mu} \qquad \text{(Eq. 10.20)}$$

where, μ = absolute fluid viscosity (kg/m s)

Water levels for the fish culture tank and the drum filter with respect to the swirl water surface were determined for a range of possible system flow rates (Table 10.18; Table 10.19). Minor pipe losses (elbows, bends, tees) were not included in the analysis. The drain pipe length from the culture tank to the swirl was estimated to be 4 m. At the maximum water flow rate the difference in elevation

between a fish culture tank and a swirl separator was 0.73 cm (Table 10.18). The pipe length from the further swirl to the drum filter was estimated to be 11 m. Head loss in the drum filter was given by the manufacturer as 20.3 cm. At the maximum water flow rate the difference between the swirl and the drum filter surface levels was -25.6 cm (Table 10.19). The negative value indicates that the drum water surface level is 25.6 cm below the swirl water surface level. A wood deck will be built to give the elevation needed to the fish tanks (Fig. 10.10).

10.9.2.- Pump selection

To select a pump the maximum quantity of water, the pump head, and friction losses in the pipes must be known. The maximum quantity of water for the Recirculating Pilot system was determined in Section 10.5.3. The pump static head is the vertical distance between the low water level (the biofilter in this case) and the highest delivery point (top of the packed columns in this case). The packed columns will have a height of 1 m measured from the bottom of the fish tank (Section 10.7.1). Therefore the water discharge from the pump will be at 1.0 m from the bottom of the culture tank and the static head from the surface at the biofilter to the top of the packed column is ~1.3 m. The system piping layout as well as length and diameters are shown in Fig. 10.11 and Table 10.20. The total head loss in the piping and fittings is 0.97 m calculated as described above (Table 10.20). Hence the total head is 2.2 m for a total flow of 32 m^3/h.

Table 10.18.- Determination of the water surface levels (Z_2) in the tanks. Swirl water surface level was used as a reference (Z_1).The analysis considered the range of water flow rates that will be used (Table 10.5).

Tanks #	Flow per tank m^3/h	Flow per tank L/min	Velocity in pipe m/s	R_e	Blasius factor	Darcy Weisbach m	Z_2 Cm
1	1.8	29	0.06	5853	0.03613	0.00026	0.04
2	1.8	29	0.06	5824	0.03617	0.00025	0.04
3	1.7	29	0.06	5795	0.03622	0.00025	0.04
3	2.3	39	0.08	7688	0.03375	0.00041	0.07
3	2.9	48	0.10	9562	0.03196	0.00060	0.11
4	2.6	43	0.09	8562	0.03285	0.00050	0.09
4	3.0	50	0.10	9940	0.03165	0.00064	0.12
4	3.4	57	0.11	11303	0.03065	0.00081	0.15
4	3.8	63	0.13	12652	0.02980	0.00098	0.18
4	4.2	70	0.14	13988	0.02906	0.00117	0.22
4	4.6	77	0.16	15309	0.02841	0.00137	0.26
4	5.0	83	0.17	16618	0.02783	0.00159	0.30
4	5.4	90	0.18	17912	0.02731	0.00181	0.35
4	5.8	96	0.20	19194	0.02685	0.00204	0.40
4	6.2	103	0.21	20462	0.02642	0.00228	0.45
4	6.5	109	0.22	21717	0.02603	0.00253	0.50
4	6.9	115	0.23	22959	0.02567	0.00279	0.56
4	7.3	121	0.25	24188	0.02534	0.00306	0.61
4	7.6	127	0.26	25404	0.02503	0.00333	0.67
4	8.0	133	0.27	26607	0.02474	0.00361	0.73

Seawater density at temperature of 20 °C and salinity of 30 g/L is 1021 kg/m^3 (Creswell, 1993).

Seawater absolute viscosity at 20 °C and 30 g/L is 0.001062 kg/m s (Creswell, 1993).

Pipe from raceway towards swirl is PVC Sc 40 of 10 cm (4 in) nominal size and 4 m length. Real inside diameter is 10.23 cm (Harrington Industrial Plastics Inc.)

Table 10.19.- Determination of the water surface levels (Z_2) in the drum filter. Swirl water surface level was used as a reference (Z_1). The analysis considered the range of water flow rates that will be used (Table 10.5). The analysis included the head loss in the drum filter, which was given by the manufacturer as 20.3 cm.

Tanks #	Flow per tank m^3/h	Total flow m^3/h	Total flow L/min	Velocity in pipe m/s	Re	Blasius factor	Darcy Weisbach m	Z_2 Cm
1	1.8	1.8	29	0.06	5853	0.03613	0.00070	-20.4
2	1.8	3.5	58	0.12	11648	0.03042	0.00234	-20.5
3	1.7	5.2	87	0.18	17384	0.02752	0.00472	-20.6
3	2.3	6.9	116	0.23	23063	0.02564	0.00774	-20.8
3	2.9	8.6	144	0.29	28685	0.02428	0.01133	-21.0
4	2.6	10.3	172	0.35	34250	0.02323	0.01545	-21.2
4	3.0	12.0	199	0.40	39759	0.02238	0.02006	-21.5
4	3.4	13.6	226	0.46	45211	0.02167	0.02512	-21.8
4	3.8	15.2	254	0.51	50608	0.02107	0.03061	-22.0
4	4.2	16.8	280	0.57	55950	0.02055	0.03648	-22.3
4	4.6	18.4	307	0.62	61237	0.02009	0.04273	-22.6
4	5.0	20.0	333	0.68	66470	0.01968	0.04932	-22.9
4	5.4	21.5	359	0.73	71650	0.01931	0.05624	-23.2
4	5.8	23.1	385	0.78	76775	0.01898	0.06347	-23.6
4	6.2	24.6	410	0.83	81848	0.01868	0.07099	-23.9
4	6.5	26.1	435	0.88	86868	0.01841	0.07878	-24.2
4	6.9	27.6	460	0.93	91836	0.01815	0.08683	-24.6
4	7.3	29.1	485	0.98	96752	0.01792	0.09513	-24.9
4	7.6	30.5	509	1.03	101616	0.01770	0.10366	-25.2
4	8.0	32.0	533	1.08	106429	0.01750	0.11240	-25.6

Seawater density at temperature of 20 °C and salinity of 30 g/L is 1021 kg/m^3 (Creswell, 1993).

Seawater absolute viscosity at 20 °C and 30 g/L is 0.001062 kg/m s (Creswell, 1993).

Pipe from swirl towards drum filter is PVC Sc 40 of 10 cm (4 in) nominal size diameter and 11 m length. Real inside diameter is 10.23 cm (Harrington Industrial Plastics Inc.)

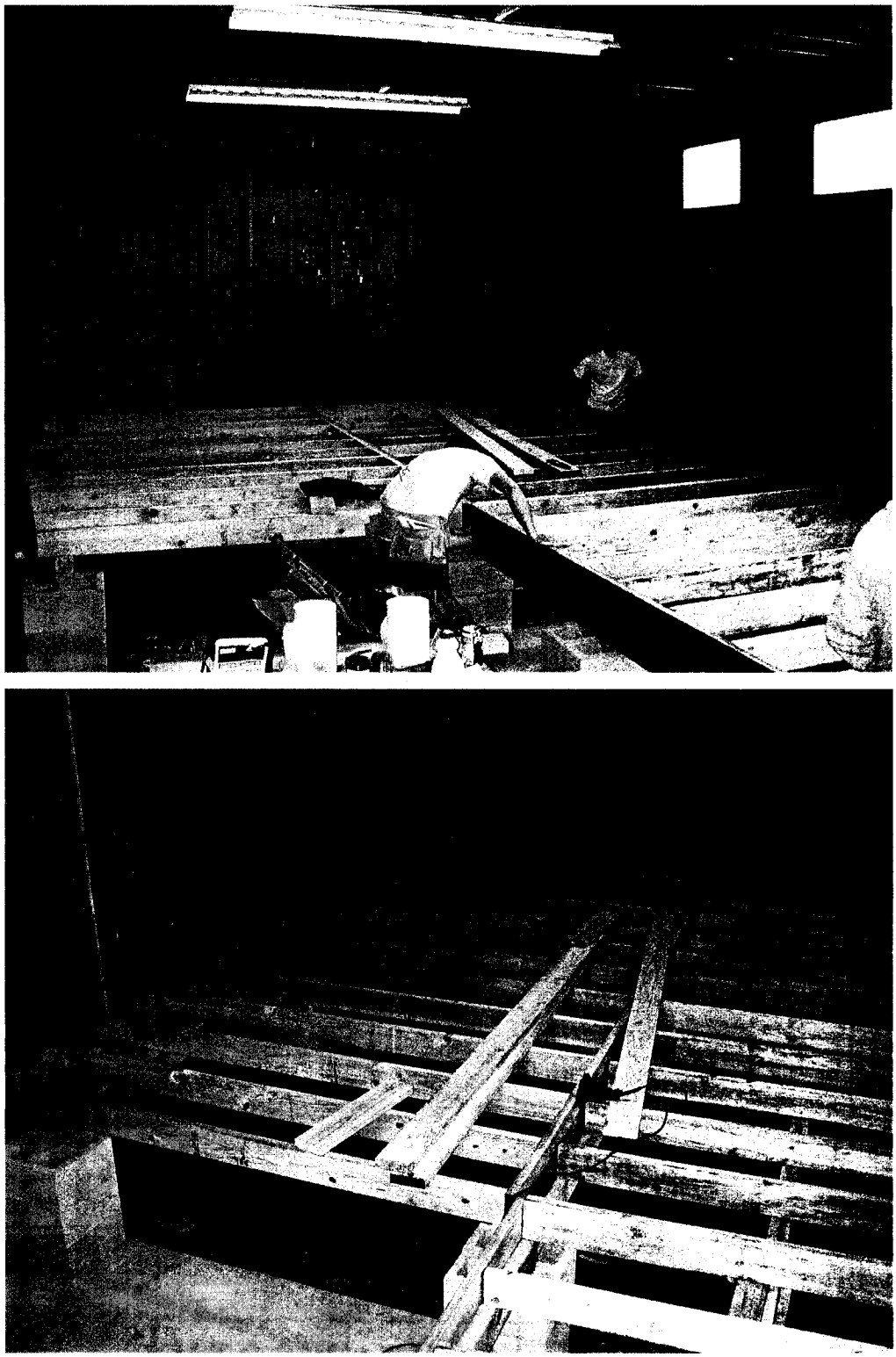

Figure 10.10.- A wood deck being built to set the fish culture tanks at levels indicated in Fig. 10.9.

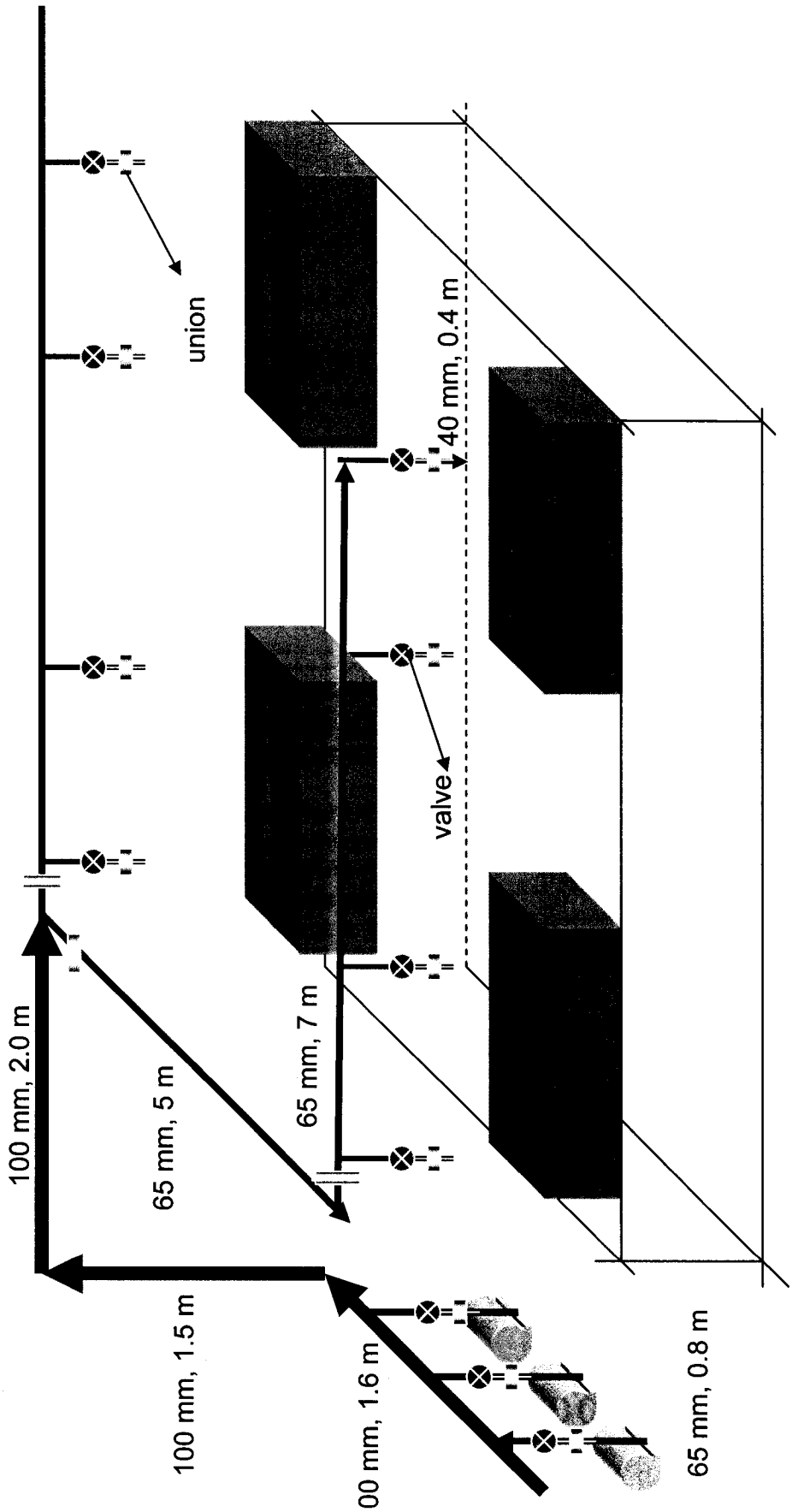

Figure 10.11.- Schematic layout of pressure piping. Drawing no to scale. Three pumps and four culture tanks will be used in the Recirculating Pilot system. The hydraulic analysis was done on the lines with arrows.

Table 10.20.- Head loss due to friction in pipes and fittings. The K values are from Larock et al. (2000).

Flow	Description	Nominal diameter	Inside diameter		Pipe aea	Velocity in pipe	Total length	Head loss pipe	K fitting	Head loss fittings
m^3/h		in	in	m	m^2	m/s	m	m		m
16	Pump line	2.5	2.47	0.063	0.0031	1.4	0.8	0.02		
	Union	2.5				1.4			0.08	0.01
	Gate valve	2.5				1.4			0.19	0.02
	Tee from 2.5" to 4"					1.4			2.00	0.21
32	Mainline	4	4.03	0.102	0.0082	1.1	5.1	0.05		
	Elbow 90°	4				1.1			0.90	0.05
	Elbow 90°	4				1.1			0.90	0.05
	Tee from 4" to 2.5"					1.1			2.00	0.12
16	Branch to first P. column	2.5	2.47	0.063	0.0031	1.4	5	0.16		
	Union					1.4			0.08	0.01
	Union					1.4			0.08	0.01
	Elbow 90°					1.4			0.90	0.10
12	1st P. column to 2nd P.	2.5	2.47	0.063	0.0031	1.1	3	0.06		
8	2nd P. to 3rd P.	2.5	2.47	0.063	0.0031	0.7	1	0.01		
4	3rd P. to 4th P.	2.5	2.47	0.063	0.0031	0.4	3	0.01		
4	4th P. to P.	1.5	1.61	0.041	0.0013	0.8	0.4	0.01		
	Elbow 90° from 2.5" to 1.5"					0.8			2.00	0.07
	Gate valve					0.8			0.19	0.01
	Union					0.8			0.08	0.00
							Total	0.31 m		0.66 m

Pumping will be provided by two pumps with an additional one on standby. This will enhance flexibility and allow routine maintenance. As noted above, the maximum flow will be delivered by two pumps; hence pumps to be chosen should be able to pump 16 m^3/h with a total a head as indicated above. Two Jacuzzi Stingray JP2 pumps (1 Hp) were selected (pump curves are available at www.aquaticeco.com).

10.10.- REMARKS

Based on the results from the Recirculating Hatchery and Nursery facilities at University of California, it is clear that California halibut can be produced under intensive conditions utilizing recirculating technology and commercially available feeds.

A recirculating system was designed to produce 1000 g fish. The system will consist of four fish culture tanks (1.5 m^3 ea); four swirl separators (one per fish tank); one drum filter; a submerged moving bed biofilter; two circulation pumps (16 m^3/h); and 10 m^3 total water volume. Aeration for the operation of the biofilter will be supplied by the Bodega Marine Laboratory facilities. A VSA oxygen generator (5 L/imin @ 90% oxygen) will also be included in the design to provide the oxygen requirement for the fish culture. This facility was designed based on the bio-engineering data presented in previous chapters.

The Recirculating Pilot system is the only one in its kind for rearing California halibut to commercial size. This recirculating system will provide a unique opportunity to collect and analyze engineering, bio-engineering, and biological data from a California halibut pilot production facility.

CHAPTER XI

GENERAL DISCUSSION

11.1.- INTRODUCTION

The primary objective of this study was to develop and establish bioengineering design criteria for California halibut recirculating rearing facilities. Hence, the purpose of this chapter is to present a discussion based on the overall advances in the intensive rearing of California halibut done at the University of California Davis, including the present research. This chapter will also discuss the relevance of California halibut as a promising species for intensive aquaculture. The previous chapter's detailed discussions will not be repeated here.

11.2.- DISCUSSION

Although California halibut is a popular commercial and sport specie in California (Caddel, et al., 1990; Gadomski et al., 1990; Hobbs et al., 1990; MacNair et al., 2001), remarkably none work on experimental or pilot productive operations have been published in order to stimulate the settlement on aquaculture commercial production on this flatfish. To start stimulating the aquaculture of California Halibut bioengineering information and factors related to the culture conditions have to be gathered. An effective aquacultural system design can be realized

only if the bioengineering criteria for design are well understood. Bioengineering data are of special concern when designing aquaculture facilities that rely on recirculating technology, particularly when sizing the water treatment devices.

Fish under intensive culture are continuously affected by farming practices (ie. handling, crowding, and stress) and fluctuating water chemistry (Wedemeyer, 1996). In devising optimal rearing strategies and culture system designs it is crucial that the stocked animal's "normal" physiological and behavioral requirements are addressed. Consequently, it is difficult to devise recommendations for intensive fish rearing solely from studies performed under laboratory conditions. However, some of these laboratory studies can be useful for preliminary decisions on the rearing environment for California halibut (e.g. Innis, 1980, Gadomski et al., 1990, and Madon, 2002). The bioengineering data gathered in this research under farm-like conditions is valuable for design purposes as it is derived in conditions that are close to those expected in a commercial production system for California halibut.

In the United States of America, the first attempts at rearing of California halibut began in the 1980s. The California halibut has been subject to experimental farming for a long time in a former Hatchery of the Natural History Museum of Los Angeles County, today the California Halibut Hatchery located at Redondo Beach (CA). Basic information gathered at this hatchery includes: spawning of captive broodstock (Caddell et al. 1990), development of early life history stages

(Caddel, et al., 1990; Gadomski et al., 1990; Hobbs et al., 1990), growth characteristics and temperature preference of juvenile stages (Innis, 1980), and intensive fry rearing at laboratory scale (Oiestad, 1995; Oiestad, 1999). At the Carlsbad's hatchery, Hubbs-Sea World Research Institute (HSWRI) also had been carried out some larval rearing and growout research (Jirsa et al. 2000; Jirsa and Drawbridge, 2001). California halibut cultured in raceways at Carlsbad's hatchery exhibited slow growth, reaching a maximum of 450 g in two years under conditions of 55-77° F (Drawbridge and Kent, 2001). Although further attempts at commercial culture were not made at the time, the results were enough to support the hypothesis of California halibut as a promising species for intensive commercial rearing.

Diverse studies were performed at the University of California at Davis toward development of a recirculating system for California halibut. As a result from studies related to the design and operation of a marine recirculating hatchery (Appendix A.1; Piedrahita et al., 2002), eggs and larvae transport (Bush et al., 2002), larvae feeding (Gisbert et al., 2004), larviculture (Conklin et al., 2002), weaning protocols (Conkling et al., 2003), and rearing techniques (Conklin et al., 2003; Merino et al., 2003), the technical feasibility of raising California halibut from egg to juvenile size in a recirculating system has been demonstrated. To be economically feasible, a recirculating system must provide and maintain the physical, chemical, and biological conditions suitable for optimum growth in high densities of California halibut. More specifically, the unit operation linked to a

recirculating system must provide and distribute life support and growth factors, collect and remove wastewater and solids from system water, reduce make-up water requirements, and recycle the water. Unit operations in an aquacultural recirculating system are linked to bioengineering variables for design and sizing. In the present work many questions related to bioengineering requirements were answered, such as water velocity, stocking density, oxygen consumption, ammonia and urea excretion, and feces and/or feed settling velocity.

In the planning of a fish culture facility, the degradation of water quality due to high fish density is a central issue in the design and operation of facilities (Pennell and McClean, 1996). Oxygen consumption and metabolite excretion rates and concentrations are related to water flow rates. Solids (ie. feces, uneaten feed) can settle within the tank if water velocities are not high enough to carry them out. However water velocity may affect fish performance (Chapter IV) and water velocity is related to tank shape and water depth.

At any aquacultural facility the production tendency will be stocking tanks to the highest density allowable. Flatfish species can be raised at high densities when adequate flow and quality of the incoming water are provided (Martinez-Tapia and Fernandez-Pato, 1991; Bjornsson, 1994; King et al., 1998; Malleck et al., 1998). In the present research, it was learned that California halibut can be reared intensively in a recirculating system. California halibut between 0.77 and 400 g can be reared at high stocking densities (up to 400% PCA) in shallow

raceways (less than 10 cm water depth), but it was found that fish growth rate is better between 100 and 200 %PCA (Chapter V).

High stocking densities will result in significant demand in the concentrations of dissolved oxygen (Timmons and Youngs, 1991; Timmons et al., 1998). Oxygen is a critical production variable which depends upon fish physiology and feed composition. Knowledge on oxygen consumption rates for California halibut can allow not only increasing fish density and water reuse, but also the sizing and selection of a given oxygen supply device as it was demonstrated in Chapter X (section 10.5.1 and 10.7) . Biomass consumption peaks must be balanced with oxygen inputs. The diel rhythm for California halibut resulted in maximum daily oxygen consumption rates that were between 20 to 50% greater than average daily values. The oxygen consumption relative to feed offered was between 0.31 and 1.4 g O_2/g feed. The basal fish metabolism requirements were included in this oxygen consumption due to the nature of the experiment performed.

The use of supplemental oxygen to increase the carrying capacity of a fish culture system or in the reuse of water will result in an increase in the concentration of metabolic wastes such as ammonia, urea, and suspended solids. Recirculation systems are characterized by the reuse of water with little discharge. Intensive recirculating aquaculture systems performances face major problems if biological filters for nitrification are not properly sized (Speece, 1973) and there is a need to know how much ammonia a fish excrete, the pattern of

ammonia excretion, and mean and maximum excretion rates. The ammonia excretion rate is related to the utilization of ingested nitrogen (Fivelstad, 1988). In the present work the TAN excretion was between 4.3 and 8.5 g TAN/ kg feed. Urea excreted by California halibut represented between 11 to 16% of the total daily nitrogen excreted. Hence urea excretion rate should be considered as a part of the overall TAN budget in a culture system for purposes of loading rates and biofilter sizing.

Solids settled within California halibut rearing tanks were quantified for their settling velocity. The settling of particles within a flatfish culture tank can become a serious factor affecting the health of the fish and disturbing their sight for effective feeding. In addition, if solids are not removed promptly from the system they can breakdown resulting in particles that are more difficult to remove (McMillan et al., 2003). In the present work it was found that the settling velocity of particles was related to the type of feed used rather to the size of fish. Settling velocities were between 0.01 and 10.10 cm/s, with ~80% of the solids having a settling velocity over 1.0 cm/s.

Finally, a pilot system was designed to produce 620 fish of 1000 g (620 kg fish) (Chapter X). The system consisted of four fish tanks, a swirl separator per tank, a drum filter, a submerged moving bed biofilter, a heating/chiller unit, three circulation pumps, blowers and a pure oxygen generator, two packed columns, and total water volume adjusted to 10 m^3. The filter media needed, oxygen

requirements, water flow, and bottom area of fish tank were calculated based on the results from this work, such as: maximum swimming velocity (Chapter IV), maximum stocking density (Chapter V), oxygen consumption rate of the fish (Chapter VI), TAN excretion rate (Chapter VII), solids settling velocity (Chapter VIII), and TAN oxidation rate of a well conditioned submerged moving bed biofilter (Chapter IX). Solids wastes will be removed in the system designed from each tank right after produced, and moved to the swirl separator, which is located right next to the tank. All swirl separators will converge in a single drum filter to remove smaller particles sizes. Solids wastes are thus removed from the system water as soon as they are produced. The system has a low head between rearing tanks and reservoir or biofilter unit, therefore low pressure centrifugal pumps are needed to circulate the system water. The submerged moving bed biofilter (SMBB) will be built as a plug flow reactor with a low head. The SMBB can also serve for gas exchange due to heavy aeration required for its operation.

No threshold or safe concentrations were determined for dissolved oxygen, ammonia, and suspended solids in this work. The thresholds for growth and survival were assumed to be similar to those reported for other flatfish (Alderson, 1979; Brown, 1980; Kikuchi, 1995; Person-Le Ruyet et al., 1998).

CHAPTER XII

GENERAL CONCLUSIONS

The general conclusions of the work presented here are for fish weighing between 0.77 and 270 g:

a) California halibut can be reared at water velocities equivalent to no more than 1 bl/s. Higher water velocities will compromise fish performance.

b) California halibut can be reared in a recirculating system and in shallow raceways without compromising growth rate and fish health between 100 and 200% PCA.

c) Oxygen consumption rates showed a diel cycle with maxima 20 to 50% over the mean daily values. The maximum oxygen consumption by fish mass can be expressed by $M_{day}=17.266\ W^{-0.2033}$ (g O_2 / kg fish d). Oxygen consumption relative to feed offered was between 0.31 and 1.4 g O_2/g feed.

d) TAN and urea-N excretion rates showed peaks when fed continuously over 12 h during the light phase period. Daily TAN excretion accounted for 84 to 89% and daily urea-N excretion for 11 to 16% of the total daily urea-N plus TAN excretion. Daily excretion relative to the feed offered was between 4.3 and 8.5 g TAN/kg feed and between 0.8 and 1.8 g urea-N/kg feed. Maximum hourly TAN excreted by fish was $M_{FISH\text{-}MAX}=0.0128\ W^{0.9889}$

(mg TAN / fish h). Maximum hourly urea-N excreted by fish was $M_{FISH-MAX}=0.0023\ W^{0.9881}$ (mg urea-N / fish h).

e) Settling velocities of particles were related to the type of fed offered rather than to the size of fish. About 80% of the solids have a settling velocity over 1 cm/s.

f) A submerged moving bed biofilter using Kaldnes™ and Rauschert™ media had a VNR of 61 g TAN/m^3 and a SNR of 0.10 g TAN/m^2 d. These values did not exclude nitrification processes occurring in other sections of the recirculating system.

g) A pilot system was designed based on criteria, equations, and methods derived from results obtained in this work, and from data obtained from the literature. The information and design criteria are applicable to California halibut planning, designing and operation.

h) California halibut is a promising species for intensive rearing in recirculating aquacultural systems.

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XIV APPENDIX

APPENDIX A.1

HATCHERY AND NURSERY FACILITIES

APPENDIX A.2

MORPHOMETRICS OF CALIFORNIA HALIBUT

(*Paralichthys californicus*) DETERMINED BY IMAGE ANALYSIS

APPENDIX A.3

UREA MEASUREMENT METHOD

APPENDIX

A.1

HATCHERY AND NURSERY FACILITIES

INTRODUCTION

A recirculating hatchery and a semi-closed nursery for California halibut (*Paralichthys californicus*) research were constructed and operated at the University of California at Davis and at the Bodega Marine Laboratory. these facilities will be described in the following pages.

MARINE RECIRCULATING CALIFORNIA HALIBUT HATCHERY

A recirculation system for the hatching and early rearing of California halibut was constructed and has been operated since 2001. The system has a total volume of 3.0 m^3 with a maximum recirculated flow of 60 L/min. The system has four raceways (2.41 m in length, 0.28 m in width, and 0.22 m in height; operational water depth between 5 and 10 cm; ~67 L maximum volume per raceway), a reservoir (2.4 m in length, 1.2 m in width, and 0.75 m in water depth; ~2160 L), a particle removal treatment unit (filter cartridges and felt bag), a submerged moving bed biofilter (2.08 m in length, 0.56 m in width, and 0.40 m in water depth; ~466 L), a UV light, a constant head tower (~100 L), a pump, and an

alarm and backup system (Fig. A1.1 and Fig. A1.2). The salinity is maintained between 28 and 32 g/L by the addition of dechlorinated tap water (< 1% system volume per day).

Various other rearing units can be annexed to the system for the carrying out of specific experiments. (Fig. A1.1; Fig. A1.2). From June to August, larviculture tanks (180 L each) were annexed to the system (Fig. A1.3). Other structures used for a variety of experiments also can be attached to the recirculating system (Fig. A1.4).

Water temperature in the system is maintained with an immersion chiller/heater (Frigidunits™ D1-100, 2000 W) located in the reservoir (Fig. A1.5). Water is pumped from the reservoir (Jacuzzi™ S1KTM) (Fig. A.1.6) through a 20 µm cartridge filter (Hayward Star Clear™ 320L26) (Fig. A1.6), and a UV disinfection unit (Rainbow™ QL-25) (Fig. A1.7) prior to being discharged into a 100 L Nalgene™ tank used as a constant head tank (Fig. A1.7). From that point, water flows by gravity through rotameter-type flow meters (model F-44500LH-8, Harrington Industrial Plastics Inc.) and into each of the four raceways (Fig. A1.8), or annexed system, as needed.

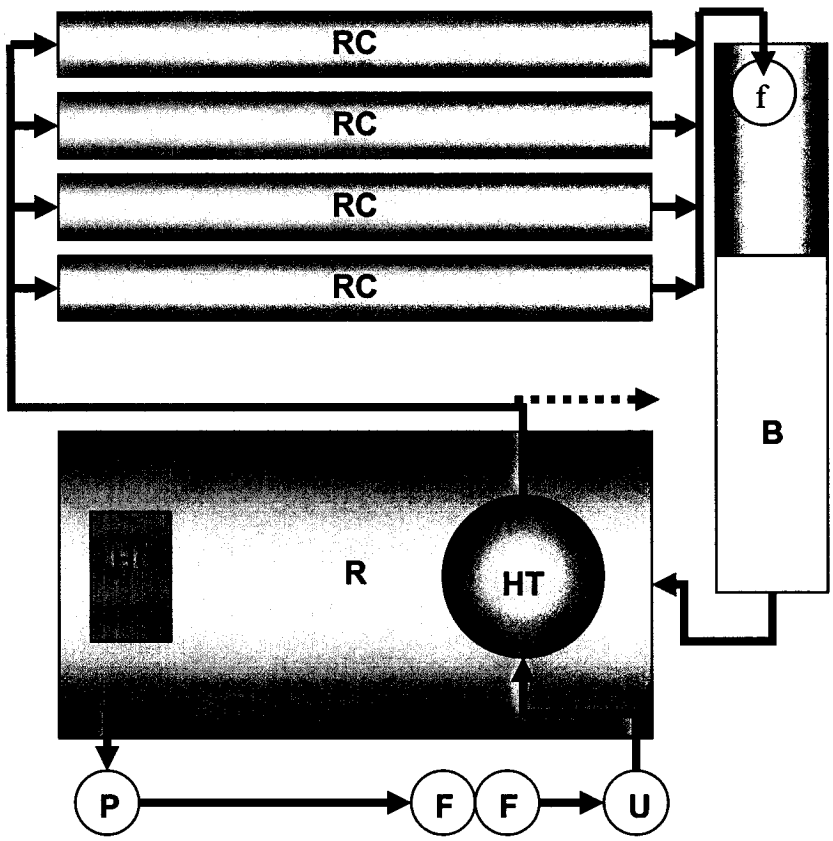

Figure A1.1.- Diagram of the experimental seawater recirculating hatchery. Total water volume is about 3000 L. R = reservoir (~2160 L); P = pump; F = filter cartridges 20 µm; U = ultraviolet light; HT = head tank (~100 L); RC = raceway (~67 L each); f = bag filter 50 µm; B = biomedia. Biofilter tank volume ~466 L. Some temporary culture units (ie. incubators) can be attached to the system, which is indicated here with the dashed arrow.

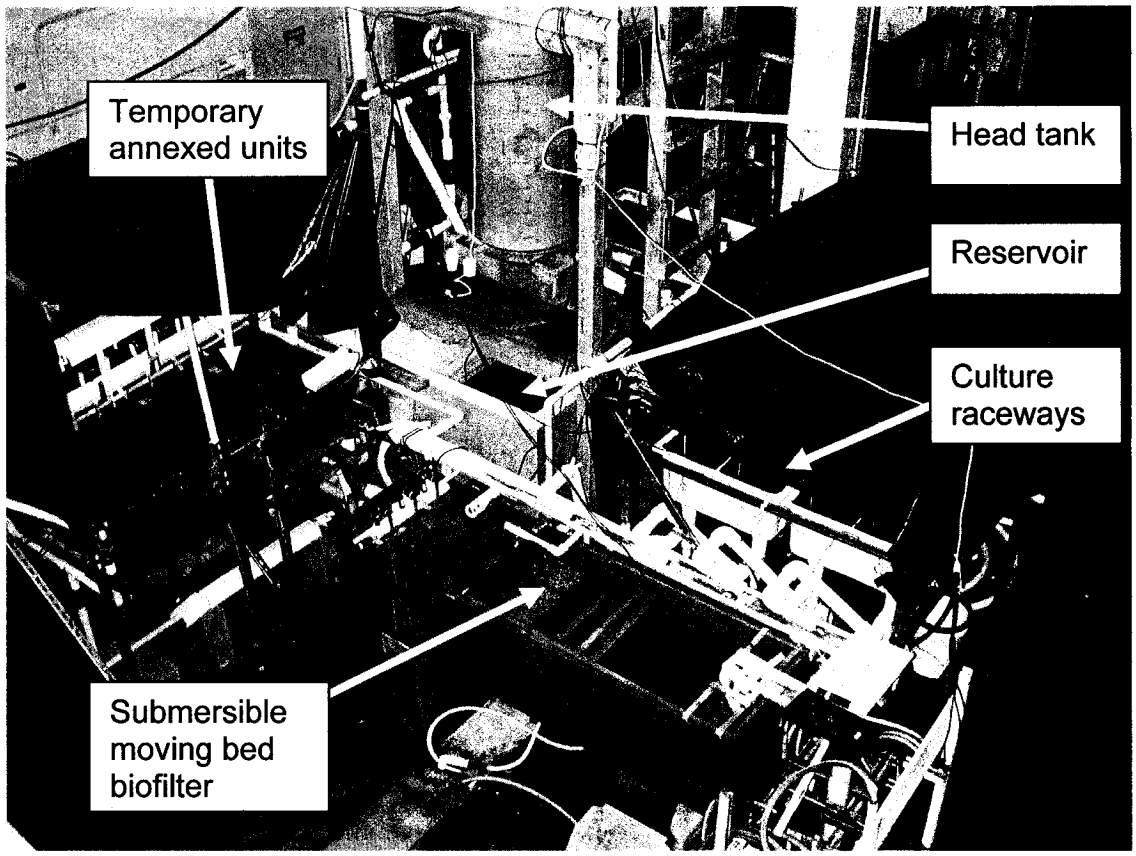

Figure A1.2.- General view of the Marine Recirculating California Halibut Hatchery located at University of California at Davis.

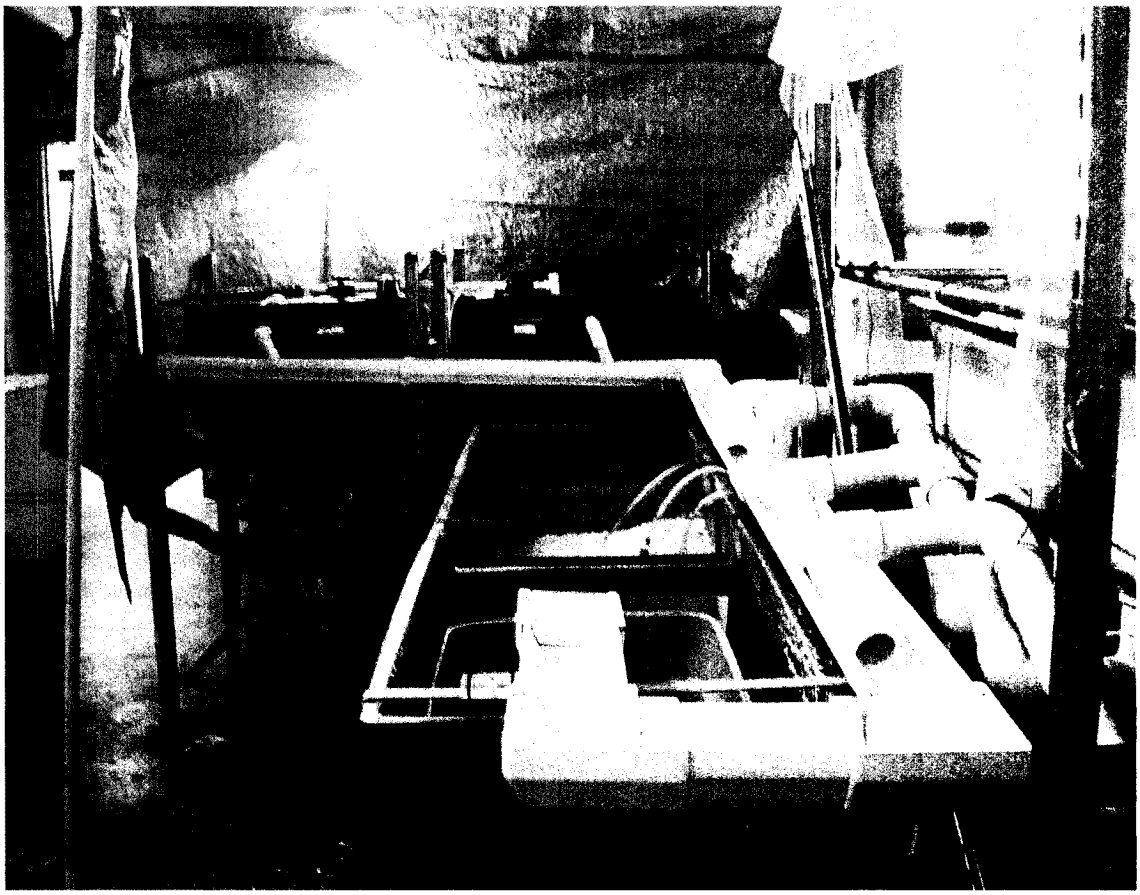

Figure A1.3.- Larviculture tanks. Two cylindro-conical tanks (black tanks in the rear) are attached to the recirculating system during the larviculture season (June-August). Influent water to larviculture tanks comes from the head tank. Effluent water from the larviculture tanks is poured into the gutter channel, where it joins the effluent water from the raceways.

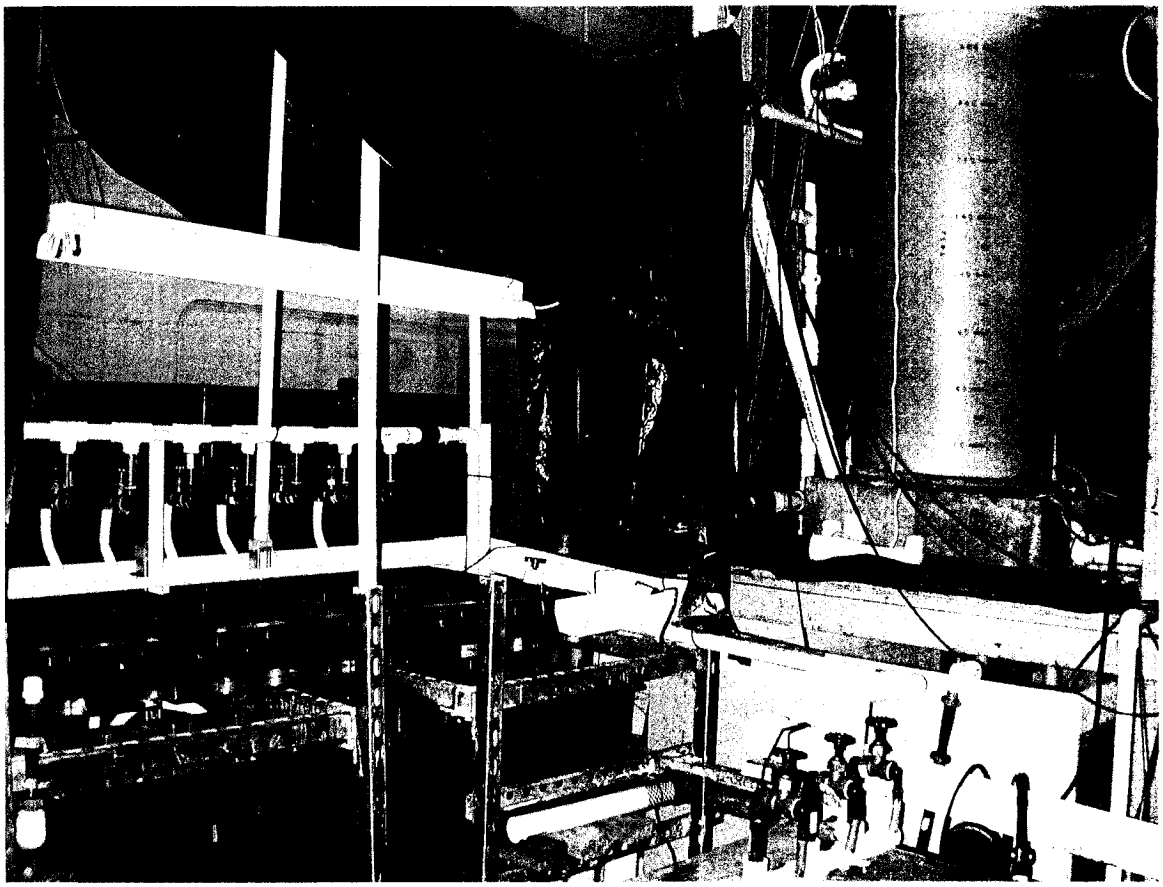

Figure A1.4.- Experimental tanks. Tanks were attached to the recirculating system to perform studies on relative swimming velocity (Chapter IV) and stocking density (Chapter V).

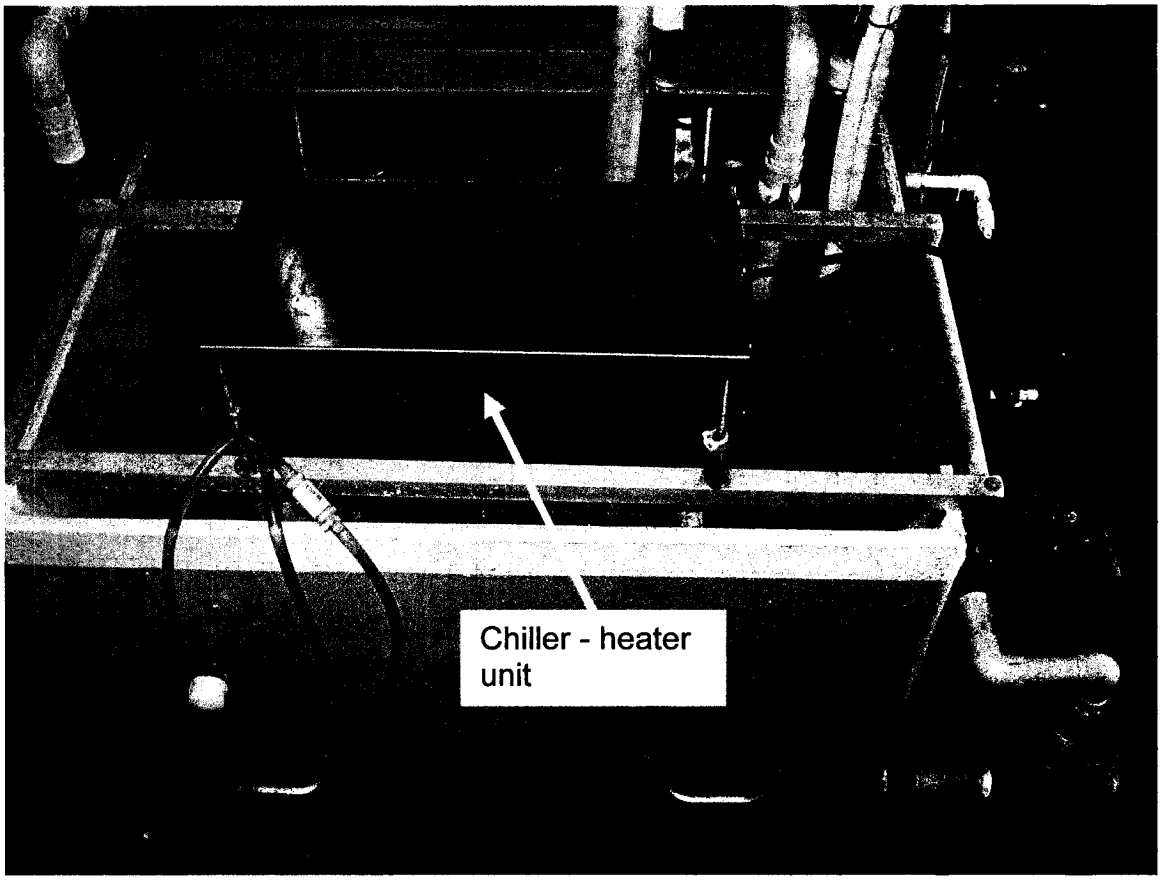

Figure A1.5.- Chiller-heater unit.

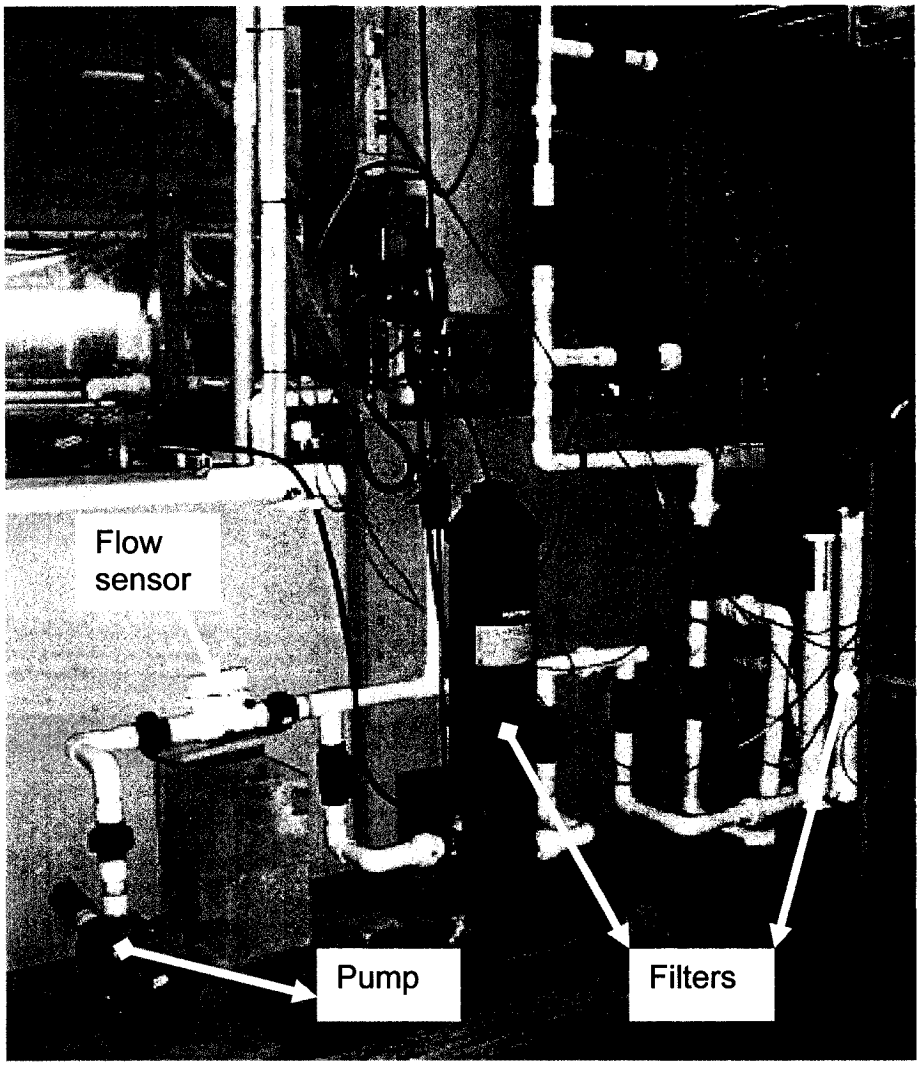

Figure A1.6.- Pump and filtration system.

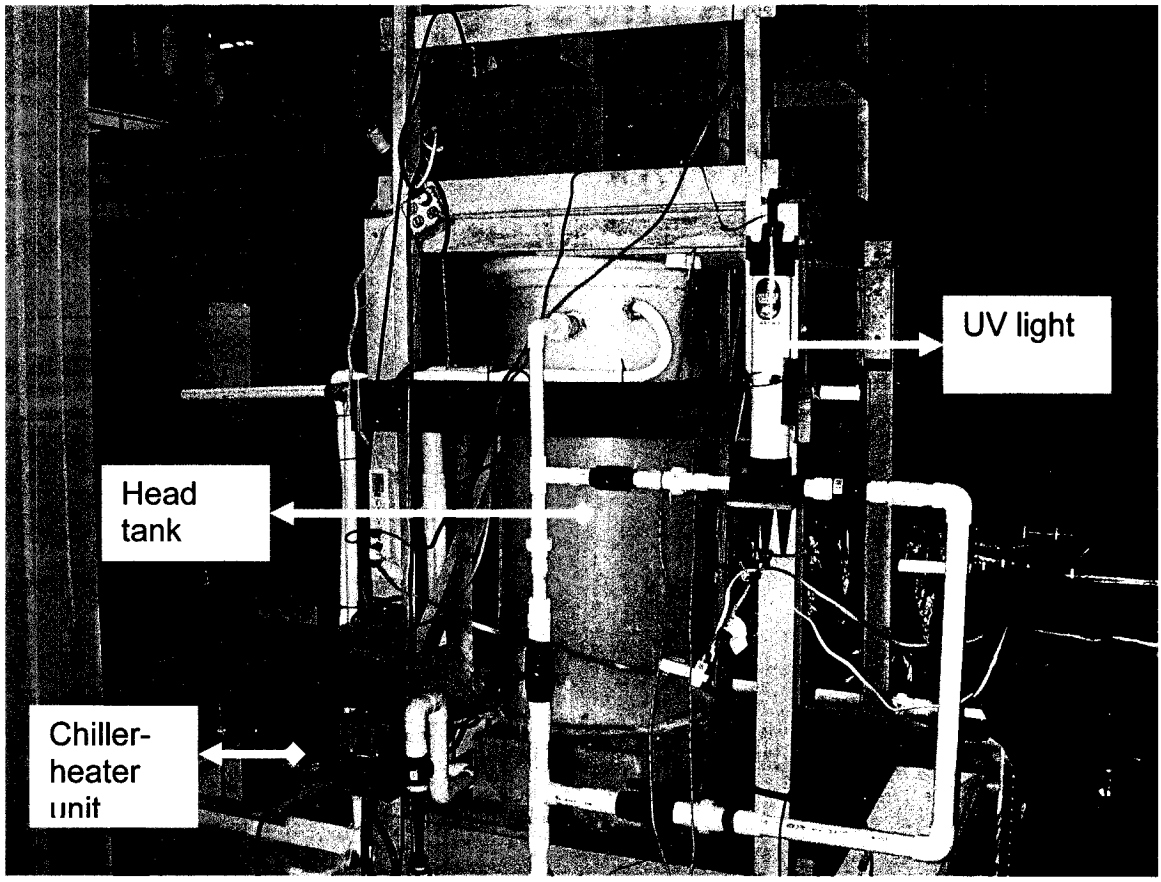

Figure A1.7.- Chiller heater unit, head tank, and UV light.

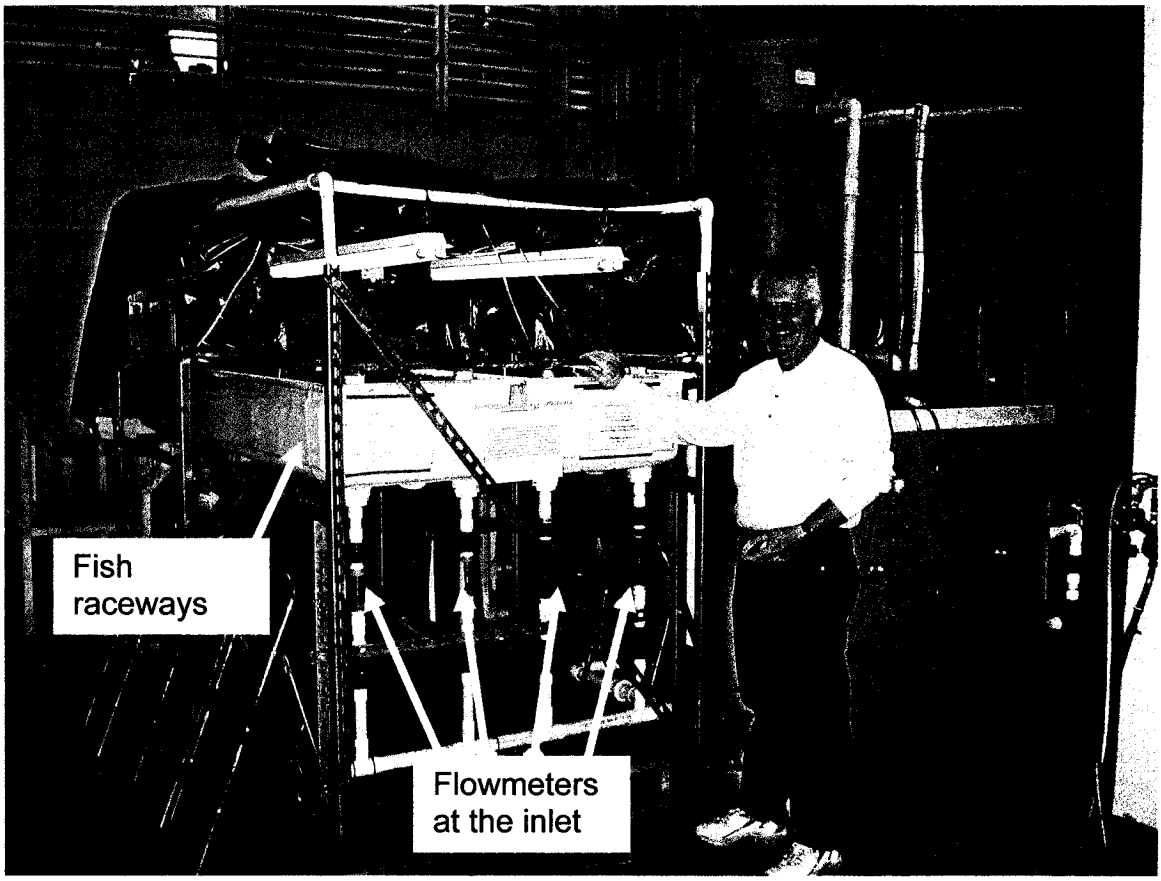

Figure A1.8.- Raceways used to rear the juvenile California halibut.

The effluents are collected on a trough and delivered to a horizontal filtration unit consisting of a felt bag of 50 µm (model FB50, Aquatic Ecosystems), a foam fractionator (0.57 m in height, 0.1 m in diameter, 0.05 m inlet/outlet, built in ABS, and with 4.5 L volume capacity), and a submerged moving bed biofilter (Fig. A1.9). Three media have been used as substrate for the bacteria in the biofilter tank (Fig. A1.10): WMT Kaldness™ 10 mm media (Table A1.1); Rauschert™ Bioflow™ 9 mm media and Biolox™ 10 mm media (Table A1.2). Aeration was provided in the biofilter unit and in the head tank from air blowing through airstones (model ASI-30, Aquatic Ecosystems).

Average oxygen concentrations are recorded every 10 min with dissolved oxygen sensors (Sensorex model DO6000) which are located at the effluent of each raceway and within the head tank. Temperature is monitored with a shielded copper-constantan thermocouple placed within the reservoir. Data from oxygen sensors and thermocouples are recorded with a micrologger (CR 21-X Campbell Scientific, Inc.) (Fig. A1.11). Data recorded with the micrologger are uploaded to a PC computer using PC208 W 3.3 datalogger support communication software (Campbell Scientific Inc.) (Fig. A1.11). Data are uploaded every week and stored in a workbook in Microsoft Excel 2002 SP-2.

An alarm system using a Sensaphone™ 1104 autodialer is used to monitor high and low water levels in the head tank and reservoir (Liquid level switch, model ST3M, Aquatic Ecosystems), flow from the pump (Flow switch, model ST11, Aquatic Ecosystems), and electrical power (Fig. A1.11).

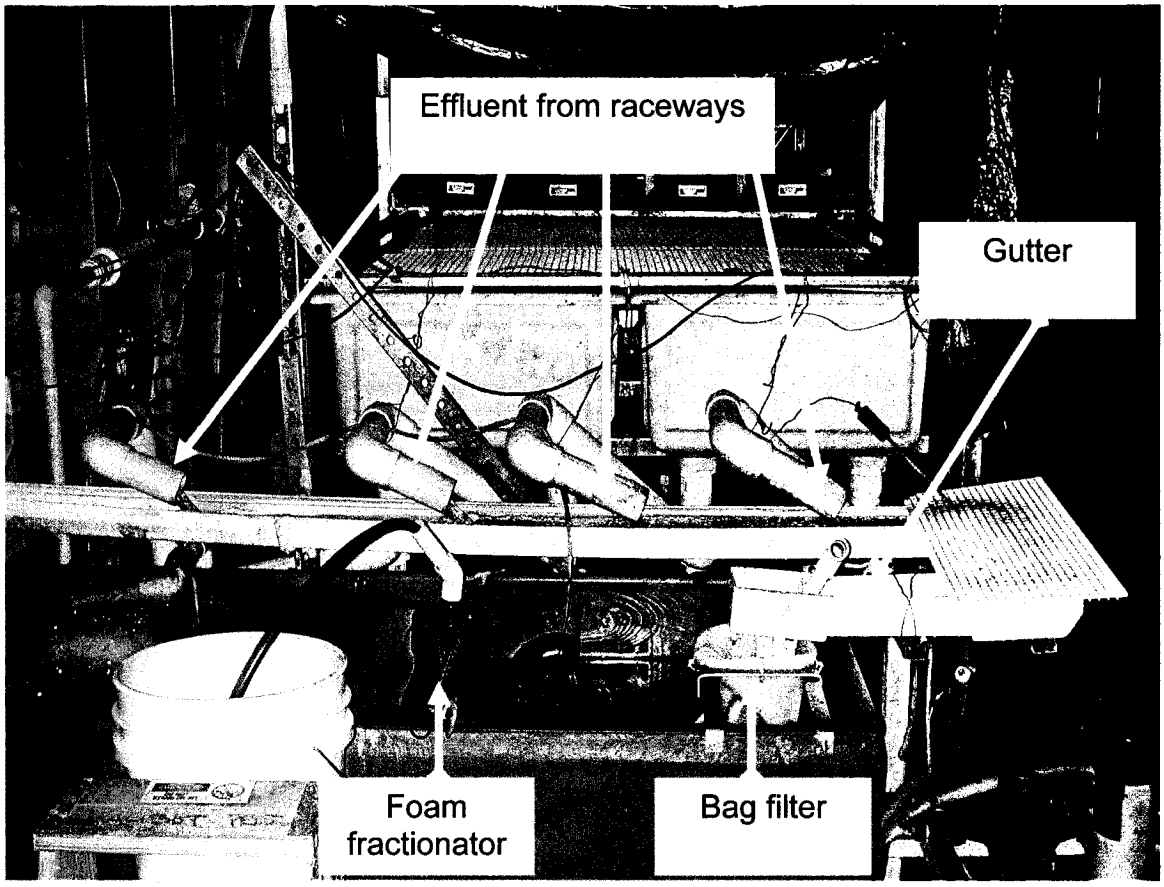

Figure A1.9.- Effluent water from system. Effluent from raceways is poured into a gutter and directed towards a felt bag filter. After the water has been filtered, part of it goes into a foam fractionator and the major part moves through the biofilter media.

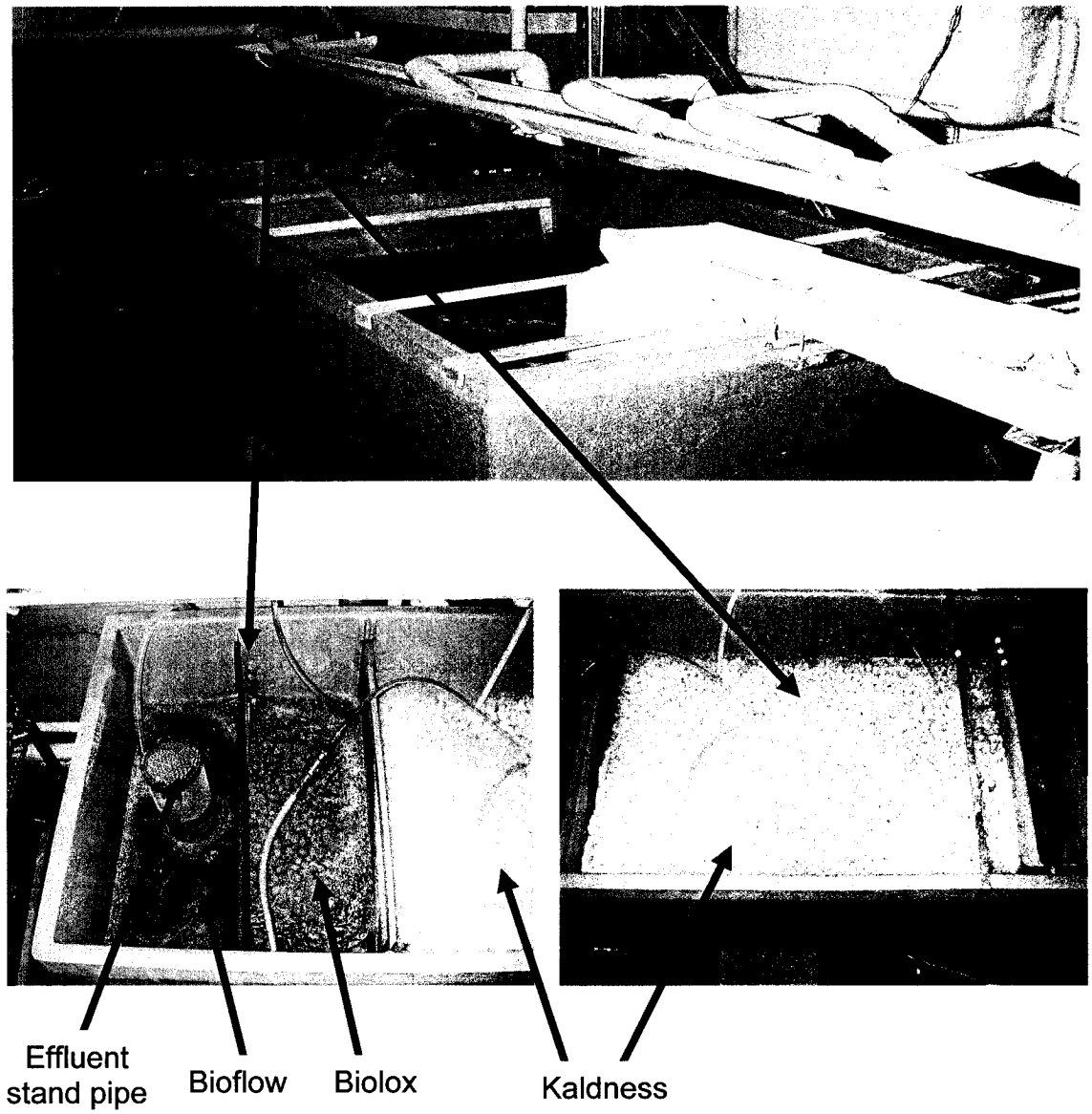

Figure A1.10.- Submerged moving bed biofilter. Floating media biofilter: KMT Kaldness™ 10 mm; Rauschert Bioflow™ 9 mm and Biolox™ 10 mm. The effluent section and hoses delivering air into the biofilter, through airstones, are also shown here.

Table A1.1.- Technical specifications for Kaldness™ filter media (http://www.W-M-T.com)

Specifications	Kaldness™
Material	Polyethylene
Specific surface area	500 m^2/m^3
Maximum fill	up to 70%
Weight	152 kg/m^3
Number of units per m^3	1,029,000
Surface per unit	4.86 cm^2
Percentage of hollow space	93%
Specific gravity	0.96
Color	natural white

Table A1.2.- Rauschert's Biolox and Bioflow rings. These rings can be supplied with material densities adjusted from 0.95 – 1.15 g/cm^3. Manufacturing material could be PP, PE, PE/PP, recycled. (http://www.rauschertus.com).

Media Type	Media Size		Media Weight		Specific Surface Area		Void Space	Specific gravity
	in.	mm	lb / ft^3	kg / m^3	ft^2 /ft^3	m^2 /m^3	%	
Bioflow 9	3/8	9	10.6	170	261	855	85	1.15
Biolox 10	3/8	10	11.2	180	195	640	82	1.09

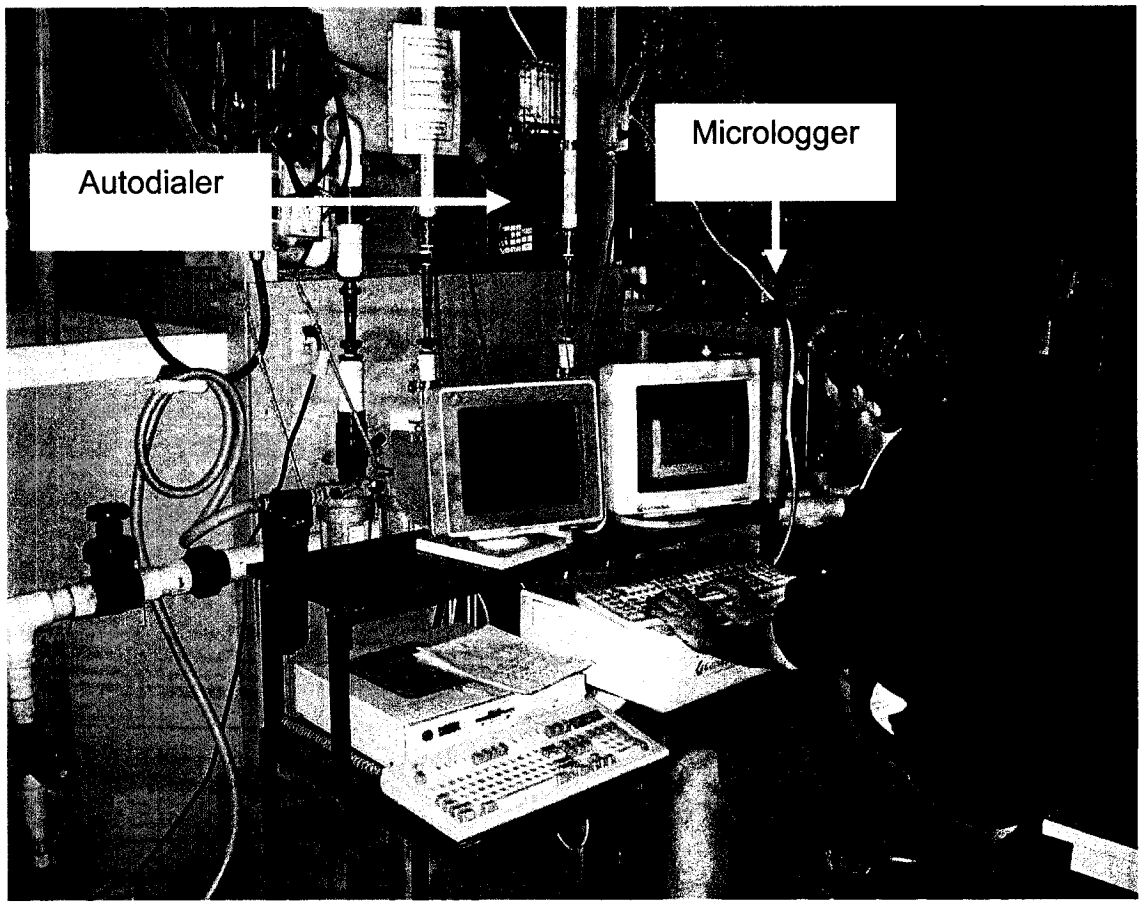

Figure A1.11.- Computer-datalogger system and autodialer alarm. An operator is downloading dissolved oxygen and temperature data stored in the micrologger. An autodialer, as a part of the sensor-alarm system, is always on to insure the safety of the fish reared in the recirculating system.

In the event of an electrical power failure a back up power system (Lithonia Lighting® battery, model ELT125, capacity 125W/12V) with a built-in battery charger, can switch on a 12 volt DC pump (Rule™ 2000 pump) that will operate for approximately 70 minutes (Fig. A1.12; Fig. A1.13). This allows for an operator to respond to the alarm telephone call from the autodialer and hand-start a gasoline powered generator (Honda™ EG2200X) (Fig. A1.14).

The seawater used as make-up is trucked in (Fig. A1.15) from the Bodega Marine Laboratory (about 160 km away from Davis) and stored in an outdoor 2000 L Nalgene™ tank at Davis (Fig. A1.16). In the holding tank the seawater is chlorinated (with 150 mL of commercial bleach per m^3) for 24h and de-chlorinated with sodium thiosulfate prior to use, as needed. Seawater stored in the outdoor tank is kept under constant aeration and filtration (filter cartridges 10 and 5 µm in series) (Fig. A1.16). About 30 L/d of seawater are discharged at 9:00h and 17:00 h from the experimental recirculating system and replaced with seawater from the holding tank. This constitutes a water exchange rate of less than 1% of system volume per day.

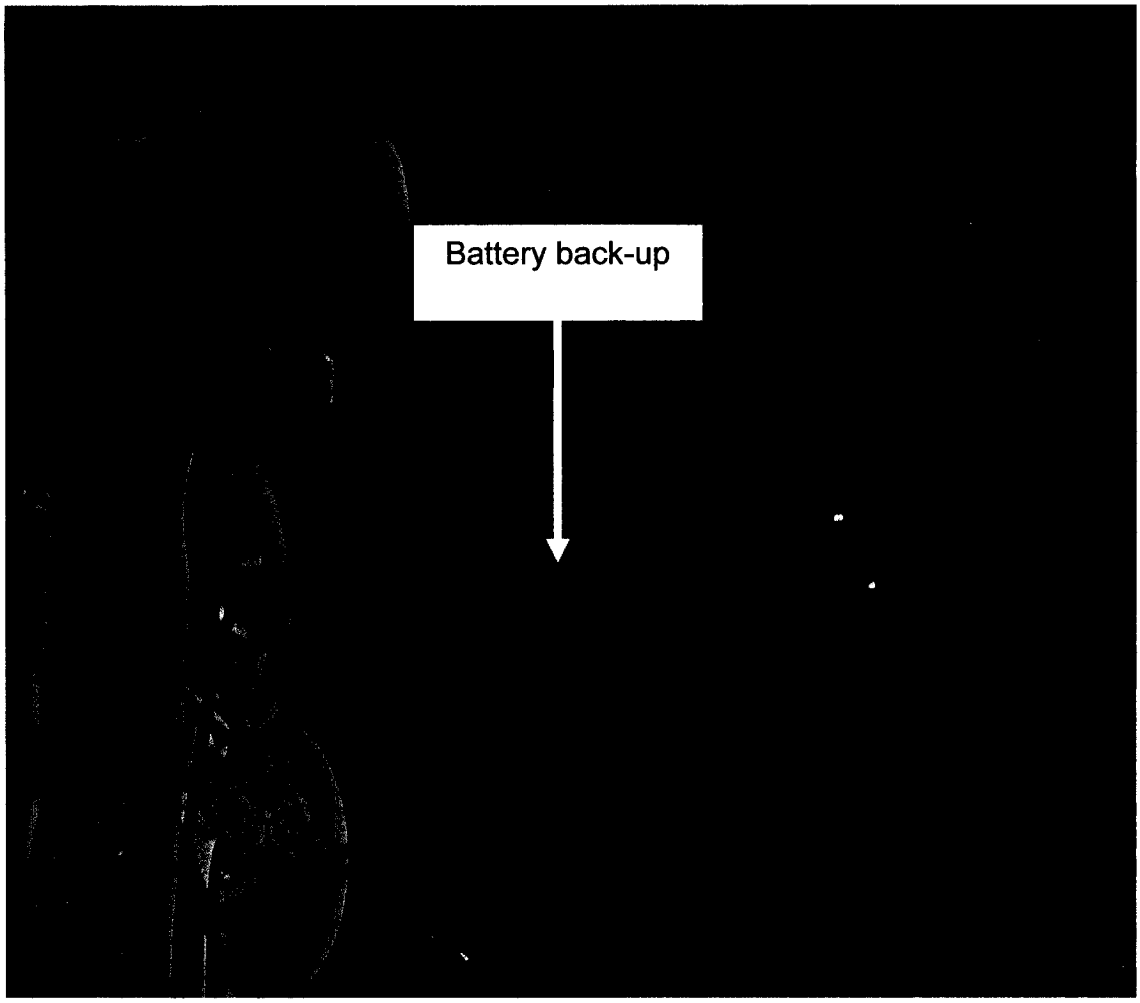

Figure A1.12.- Lithonia Lighting® battery system. The battery will power up an emergency pump located in the reservoir in case of an electricity failure.

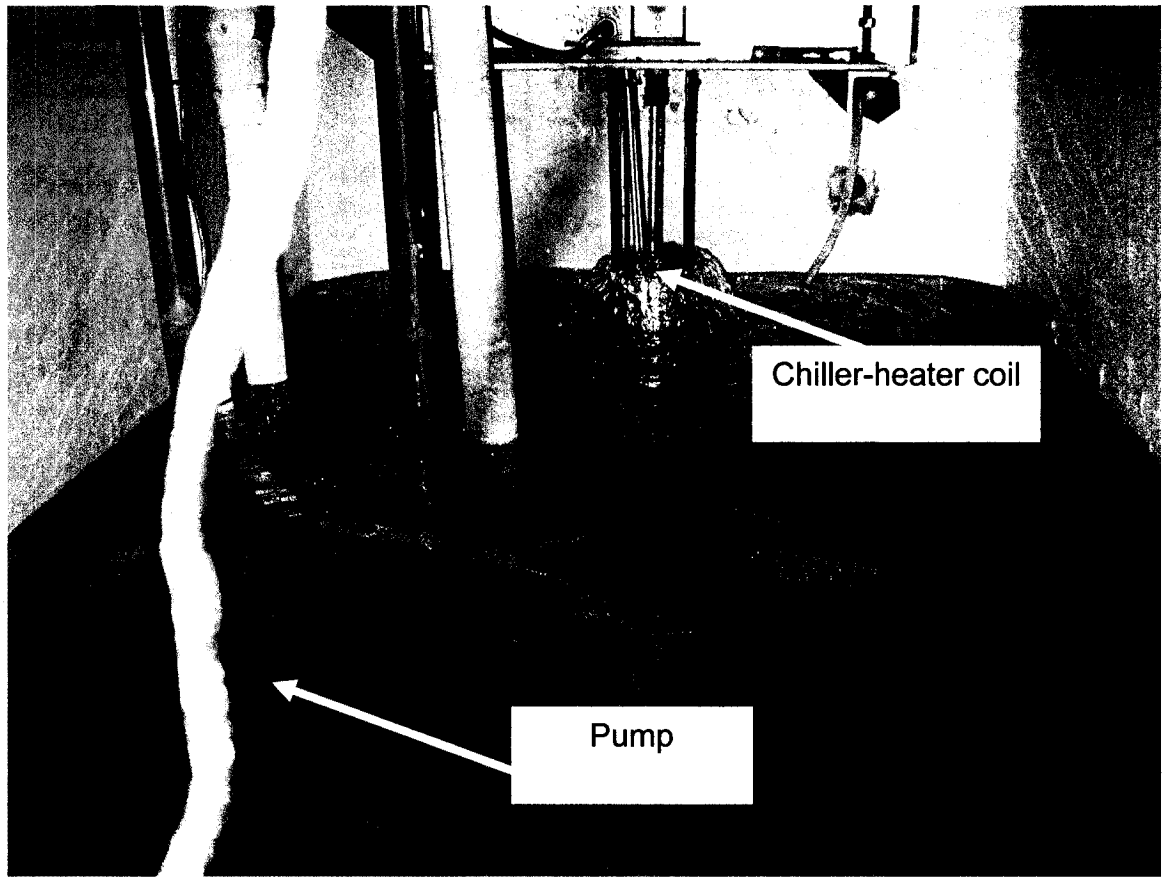

Figure A1.13.- Battery powered pump. The auxiliary pump is submerged within the reservoir.

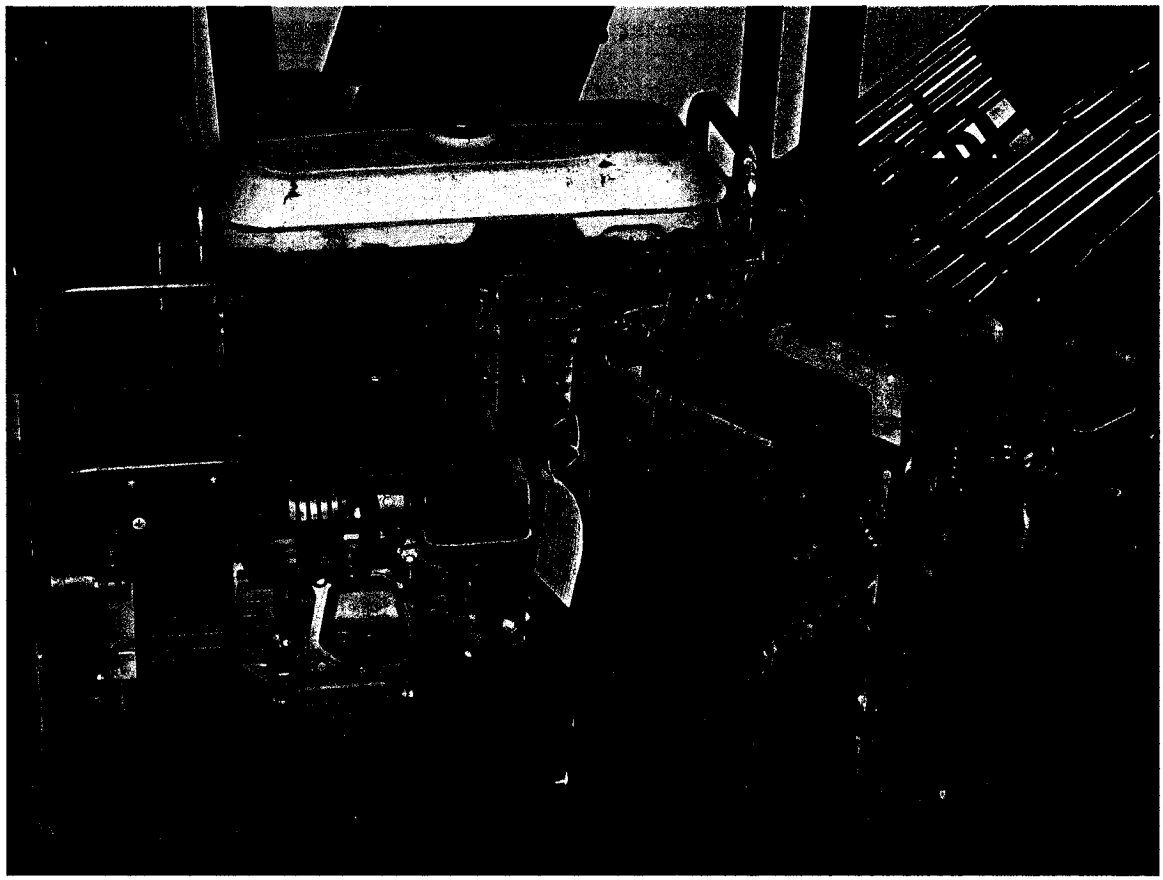

Figure A1.14.- Gasoline powered generator. More than one generator unit is held on stand-by in case of an electrical power failure.

Figure A1.15.- Seawater loaded on the truck at the Bodega Marine Laboratory.

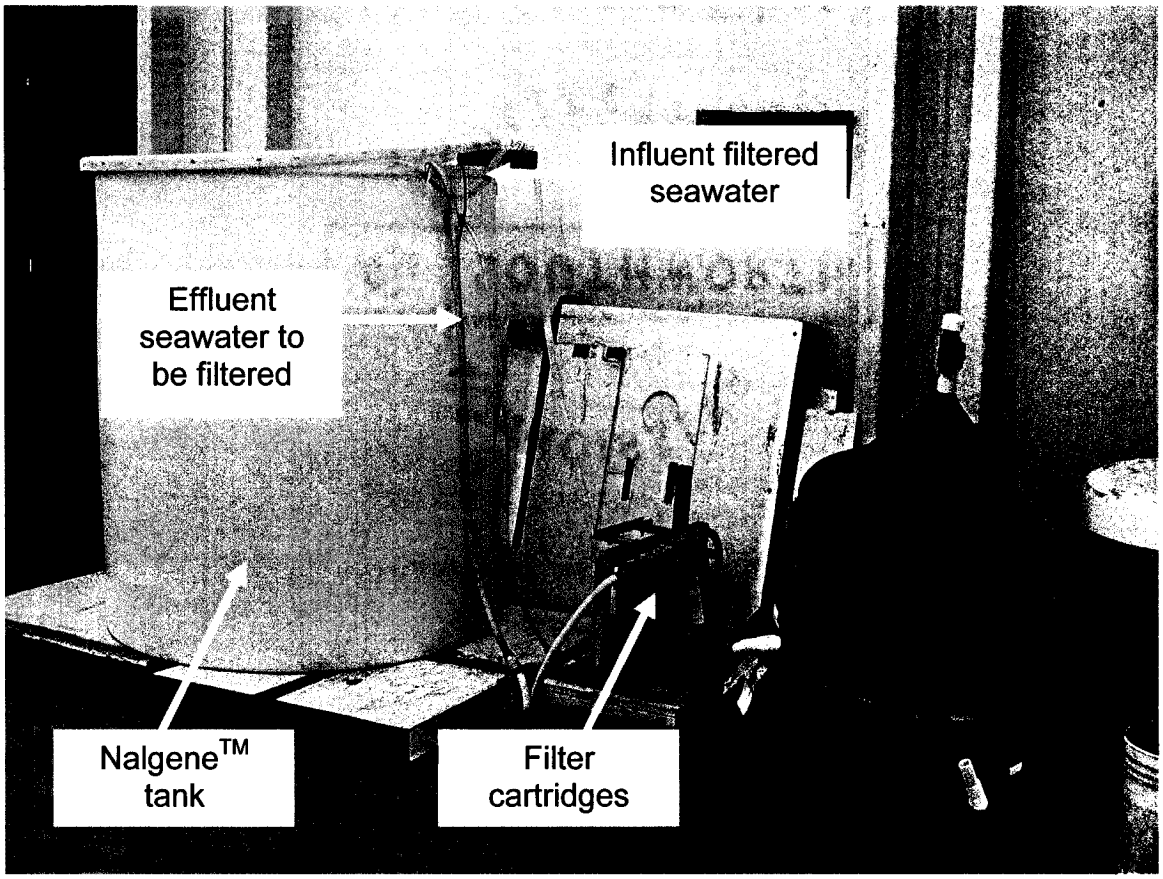

Figure A1.16.- Stored seawater to be used as make-up. Stored water is continuously filtered and aerated.

General system maintenance consists of:

a) Cleaning raceways once a day in the morning. Raceways were cleaned daily in the morning and before feeding. The cleaning process consisted in manually scraping the tank bottom and submerged walls, from influent to effluent section, to remove biofouling and dead fish (Fig. A1.17).

b) Cartridge filters are installed in the morning and removed in the evening. Immediately after their removal, the cartridges are cleaned with pressurized tap water, and left submerged in a bleach solution overnight. The system is left without cartridge filtration overnight.

c) The bag filter is replaced twice per day. After removal, the bag is cleaned with pressurized tap water. After cleaning with tap water, the bag is left overnight in a bleach solution. Bag filtration operates continuously.

d) Water quality parameters (pH, alkalinity, temperature) are measured daily in the morning. Ammonia and nitrite are measured once per week. Sodium bicarbonate is added twice a day for alkalinity compensation.

e) Feed is put into each of the belt-feeders on the raceways at approximately 9:00 AM.

f) Make-up seawater is added in the morning (15 L) and in the evening (15 L). A similar amount of water is removed from the system before adding the make-up water.

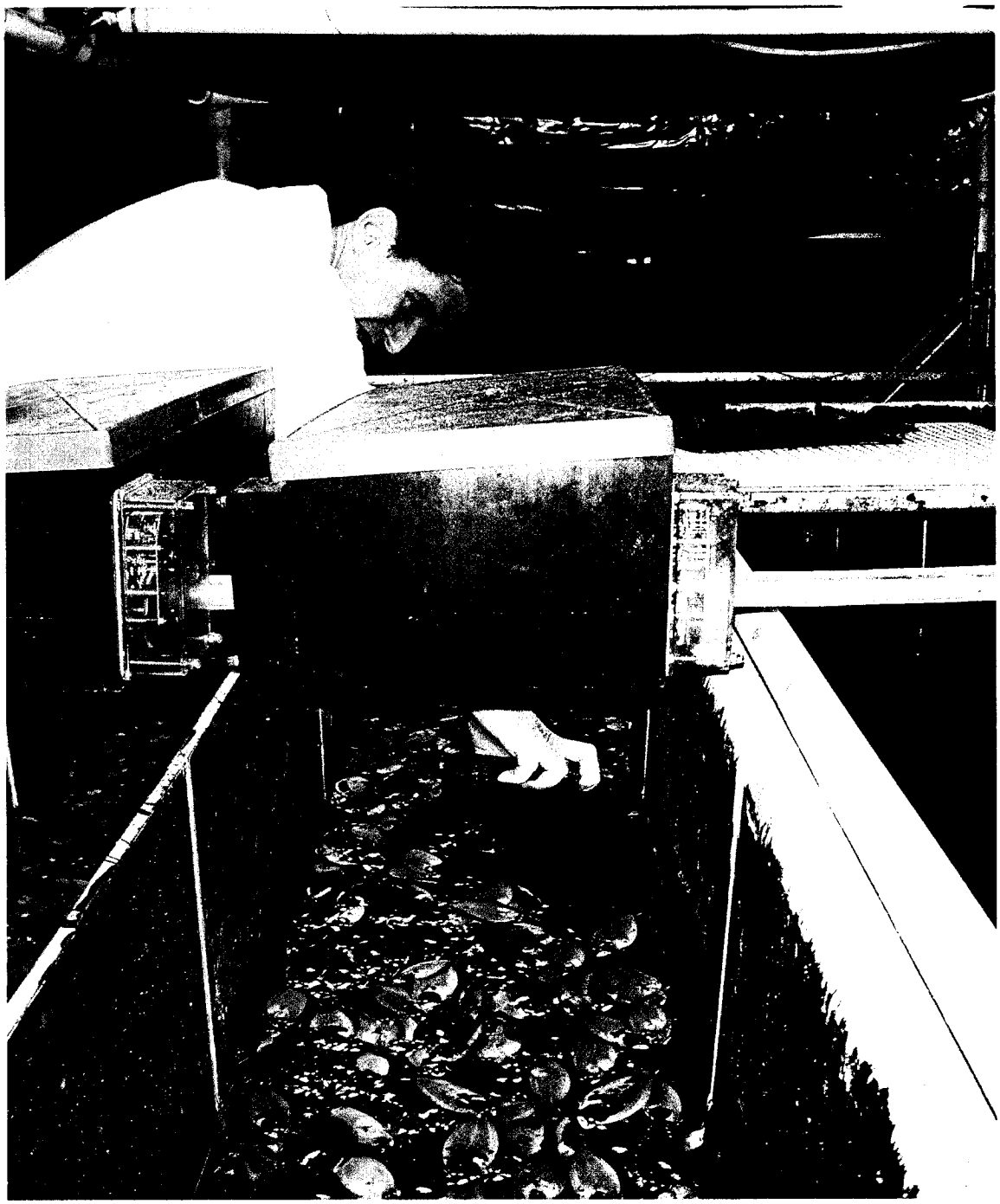

Figure A1.17.- Raceway cleaning process. Belt feeder is located over the raceways near the inlet.

g) Dechlorinated (with activated carbon) tap water is added as needed to keep a salinity of 30 g/L.

h) Data are downloaded from the datalogger to the PC once per week.

SEMI-CLOSED CALIFORNIA HALIBUT NURSERY

California halibut juveniles are moved out from the recirculating system at Davis to the nursery at Bodega Bay, when they are ~60 g average mass. Once the truck with the load of fish has arrived to the nursery facility, the fish are acclimated to the new rearing water and then transferred to the nursery tanks. The primary differences for the Bodega system from the Davis system are that circular tanks are used instead of raceways and, because of the availability of piped seawater at the laboratory, the system is typically operated in a semi-closed manner with a constant input of raw seawater. However, when there are interruptions in the laboratory's seawater supply, the system can also be operated in a closed recirculation mode. Aeration is provided from the Bodega Bay Marine Laboratory piped air system and delivered continuously to each tank through two airstones.

Water temperature in the system is maintained with a heat exchanger (titanium tube-in-shell, flowing boiler water at 70-90 ^{o}C). System water is operated in a closed loop flow (40 L/min) pumped (Jacuzzi™ S1KTM) from the head tank (Fig. A1.18; Fig. A1.19). The flow of hot water in the heat exchanger is turned on/off

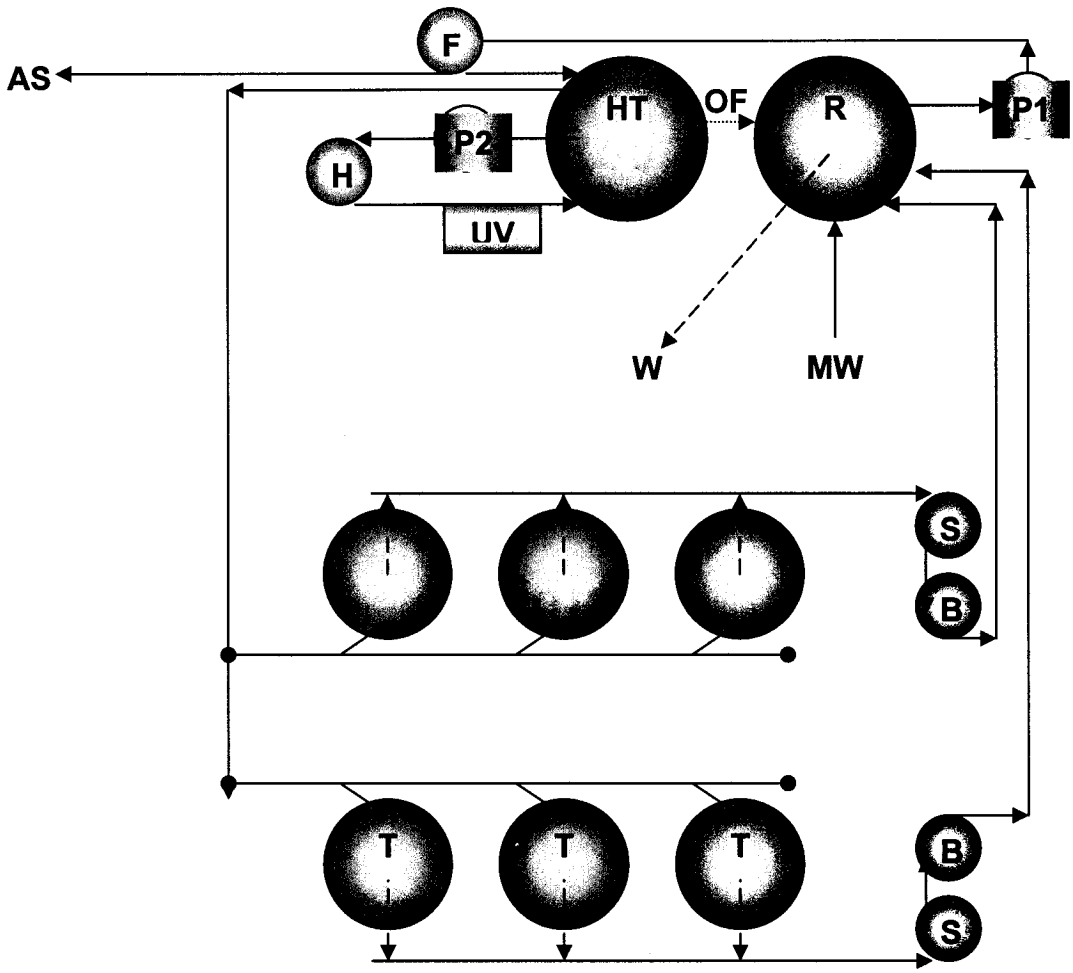

Figure A1.18.- Diagram of the experimental seawater semi-closed nursery system at the Bodega Marine Laboratory. Total water volume is approximately 3300 L. P1 = system water pump; P2 = heat-exchanger head-tank water loop pump; F = pressurized sand filter; UV = ultra violet light; HT = head tank (~800 L); R = reservoir (~400 L); T = fish tank (~300 L each) ; H = heat exchanger; S = settling tank (~80 L); B = biofilter tank (~80 L); MW = make-up water; W = overflow water from system; OF = overflow water from head tank to reservoir. Temporary culture units (AS) can be attached to the system. Arrows indicate the direction of water flow.

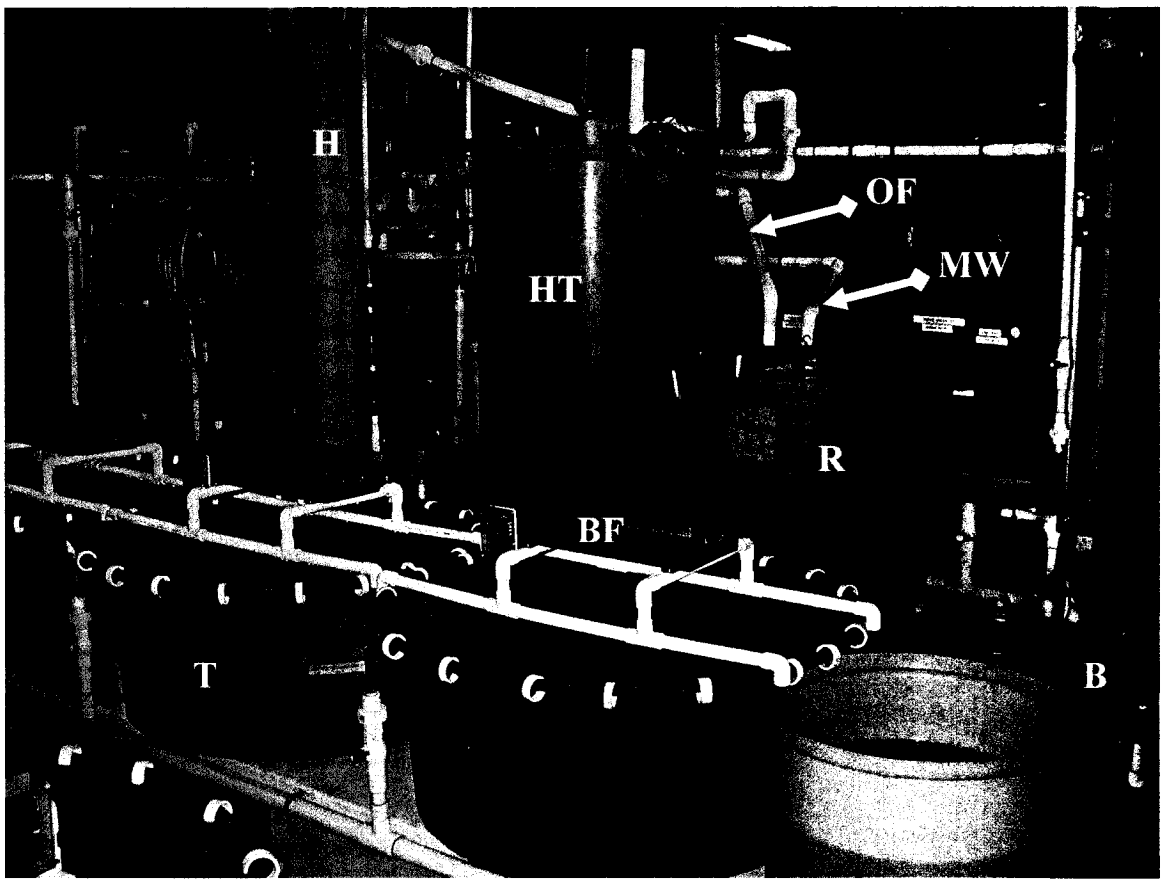

Figure A1.19.- A partial view of the nursery facility. At the back from left to right are a heat exchanger (H), head tank (HT) and reservoir (R). In front, from left to right, are three covered circular tanks (T) with their respective belt feeders (BF). At right bottom is a settling tank (S) and a biofilter tank (B). The make-up water pipe (MW) is on top of the reservoir (R). Excess water in the head tank (HT) overflows through a pipe (OF) to the reservoir (R).

by a temperature controller (Process Technology® model 92SM), which regulates the operation of a normally close solenoid valve. The temperature controller is set to maintain the water within the head tank to be 20±1 °C. The temperature controller is equipped with a vinyl-sleeved sensor tube, which is installed within the head tank.

System water is pumped (Jacuzzi™ S1KTM) from the reservoir through a sand filter (Doughboy® high rate sand filter, model # 0-1701-016 with 0.13 m^2 of filtering surface, maximum flow of 100 L/min), and a UV disinfection unit (Emperor Aquatics, model EU40, 40 Watts) prior to being discharged into a ~800 L Nalgene™ tank used as a constant head tank (Fig. A1.18; Fig. A1.19). From the head tank, water flows by gravity through rotameter-type flow meters (model F-450LHN, Harrington Industrial Plastics Inc.) and into each of the six circular tanks at a maximum flow of 12 L/min per tank (Fig. A1.20; Fig. A1.21). The excess system water from the head tank overflows into the reservoir.

An alarm system using a Sensaphone™ 1104 autodialer is used to monitor high and low water levels (Liquid level switch, model ST3M, Aquatic Ecosystems) in the head tank and reservoir, flow through the sand filter (Flow switch, model ST11, Aquatic Ecosystems), and electrical power (Fig. A1.20).

There are six circular tanks 100 cm in internal diameter and 51 cm in depth (Fig. A1.20). The water level in the culture tanks is controlled by the level in the

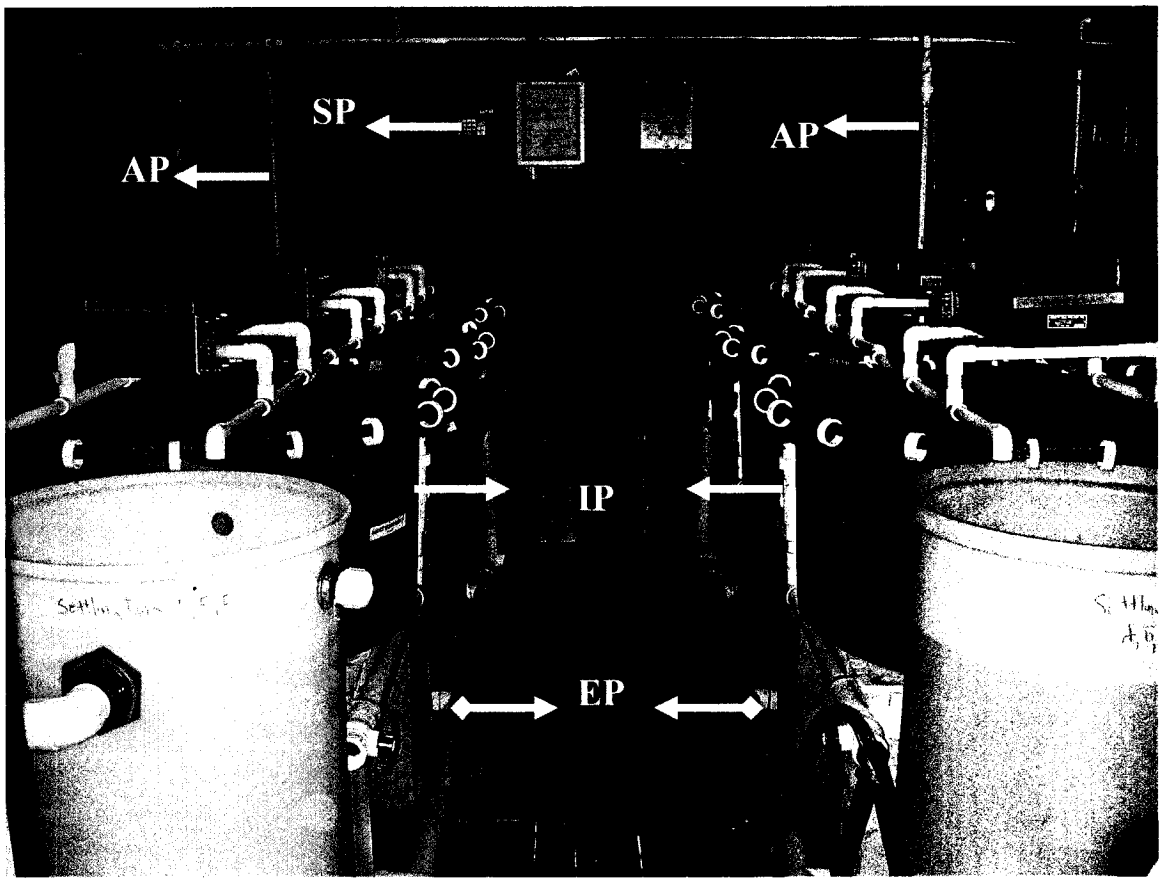

Figure A1.20.- General view of culture tanks. AP = airline pipe; IP = influent pipe; EP = effluent pipe; SP = autodialer.

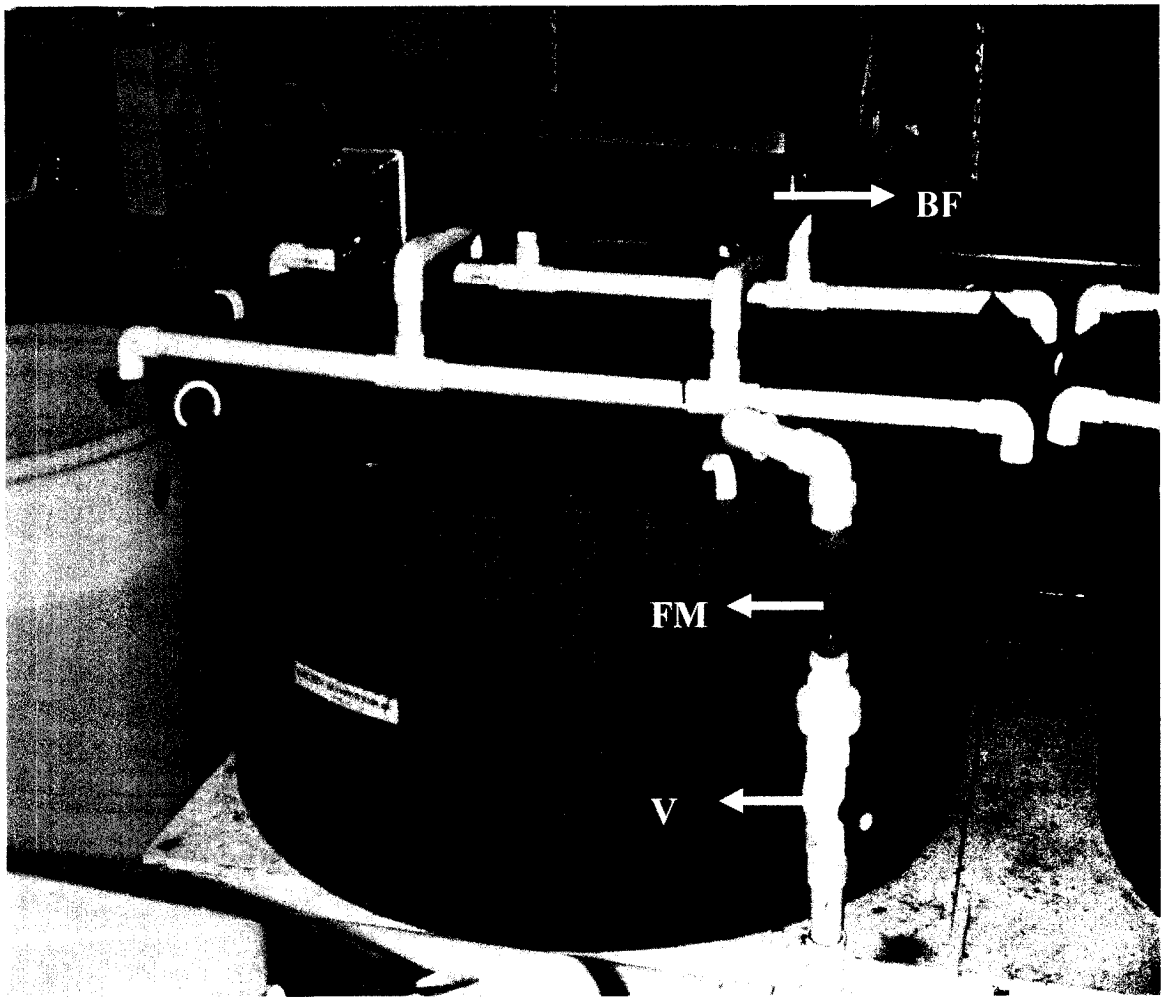

Figure A1.21.- Culture tank close up. V = ball valve; FM = flow meter; BF = belt feeder.

settling tank, which works as an external standpipe (Fig. A1.22). The biofiltration unit uses a submerged moving bed media (Fig. A1.22). The medium used is a Rauschert™ Biolox™ 10 mm medium (Fig. A1.23) (Table A1.2). Aeration is provided to the biofilter unit and each of the circular tanks from air blowing through airstones.

Make-up seawater from the BML piping system is added continuously at a rate between 2.2 and 14.4 m^3/d into the reservoir. A bag filter (50 µm) is attached to the make-up water pipe. A similar amount of water overflows from the reservoir.

Under normal operation, the general system maintenance consists of:

a) The sand filter is backwashed twice a day (morning/evening) with pressurized seawater taken from the reservoir.
b) System seawater lost during the backwash process is recovered after a few hours from the make-up water.
c) The bag filter for the make-up water is changed every 24 h. The filter removed is washed and left in a bleach solution for 24 h.
d) Feed is placed into each of the belt-feeders at approximately 9:00 AM.
e) Flow meters attached to each circular tank are cleaned every other week.
f) Particulate materials settled in the settling tank are drained every other day.

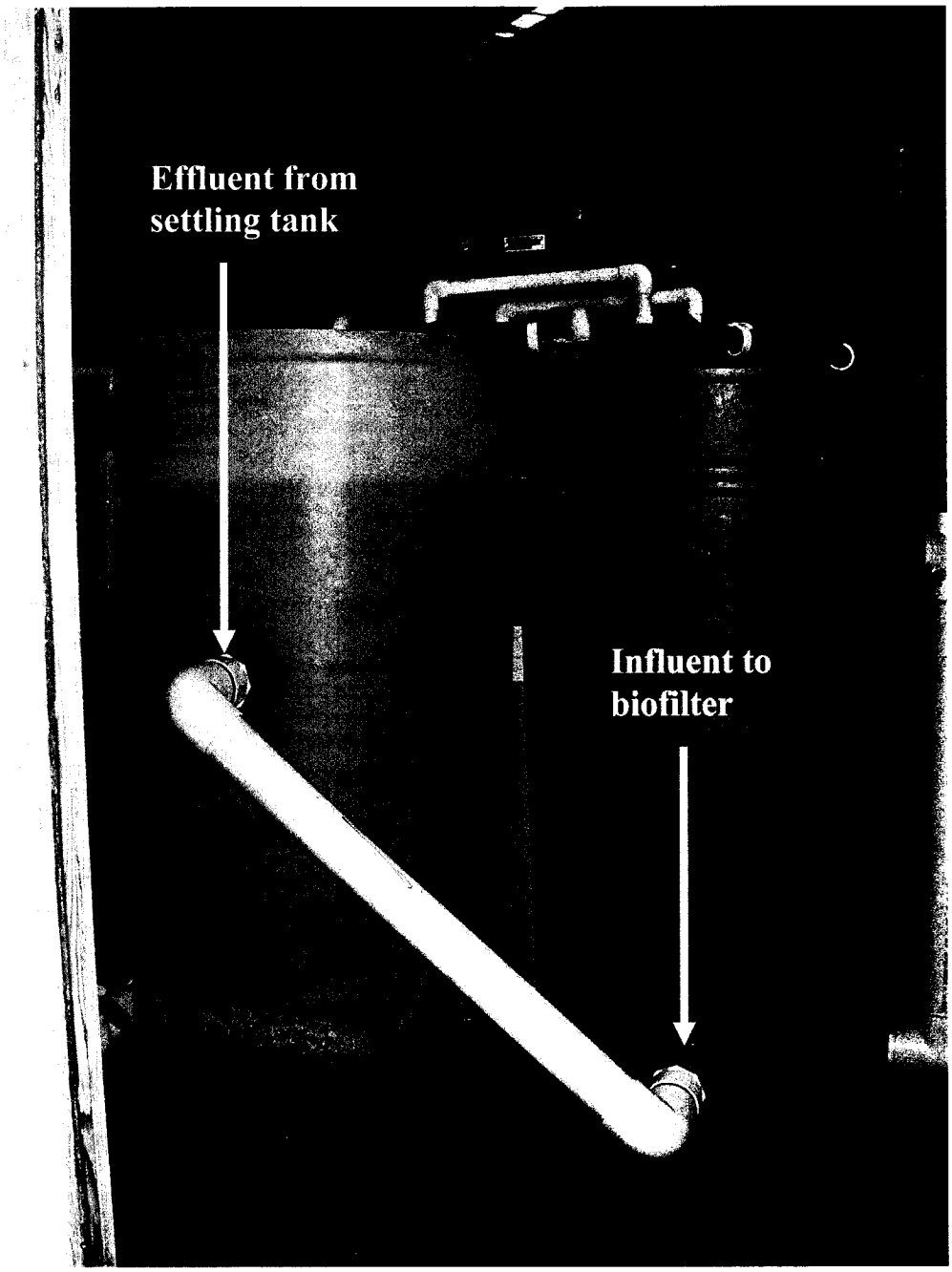

Figure A1.22.- Settling tank and submerged moving bed biofilter.

Figure A1.23.- Close up of the submerged moving bed biofilter with Rauschert™ Biolox™ 10 mm biomedia.

APPENDIX

A.2

MORPHOMETRICS OF CALIFORNIA HALIBUT (*Paralichthys californicus*) DETERMINED BY IMAGE ANALYSIS

INTRODUCTION

The California halibut (*Paralichthys californicus*) is one of the most economically important flatfish species of Southern California in the USA (Gadomski et al., 1990) and Baja California in Mexico (Hammann and Ramirez, 1990). The first attempt to culture California halibut under laboratory conditions was reported in 1990 (Gadomski et al., 1990). The following step was to determine the survival and growth rates of egg, larvae, and early juveniles exposed to different temperature environments (Gadomski & Caddell, 1991). Later efforts were directed towards the development of technologies to produce juveniles (Oiestad, 1999; Jirsa & Drawbridge, 2000). Currently there is ongoing work developing technology that will support a commercial activity in the areas of hatchery (Bush et al., 2002; Gisbert et al, 2002), nursery (Piedrahita et al, 2002), and grow out (Conklin et al, 2002).

The culture density of flatfish is frequently reported as the percentage of tank surface area covered (Bjornsson, 1994; Klokseth & Oiestad, 1999). A common

practice to estimate surface area of flatfish in aquaculture is by placing the fish over a paper grid, drawing the fish edges, and counting the area enclosed by the drawing (Bjornsson, 1994; King et al., 1998). The limits of this current methodology are numerous. Foremost is that since the process is labor intensive and time consuming only a few animals can be sampled. Sampled fish are subjected to excessive manipulation, with a high potential for injury.

Photographic and computer image analysis techniques have being used recently in fisheries research (Bates & Tiersch, 1997; Cadrin & Friedlan, 1999), mollusk ecotoxicological testing (Johnson et al., 2001), and mollusk aquaculture (Pontual et al., 1998; Vilchis, 1998). The goal in this appendix is to describe a procedure to perform routine monitoring of flatfish allometric measurements using image analysis technology.

PROCEDURES

Fish sampling protocol

California halibut juveniles have been cultured at the recirculating seawater facility at the University of California at Davis since 2001 (Conklin et al., 2002). Fish were sampled every 30 days since they were 0.1 g and 19 mm standard length. Fish were fasted 24 h before sampling for morphometric analysis. At each sampling, about 20 to 60 fish were selected randomly from each of the culture tanks. Each fish was then placed in a white container, with enough seawater to

cover the fish. A 15 cm ruler was placed next to the fish. The container was placed over a light table, to increase the fish edge contrast, and the fish and ruler were photographed using a digital camera (Nikon Coolpix 990). Immediately after being photographed, the fish was individually weighed and returned to the culture tank. California halibut juveniles are very easy to handle, hence the use of an anesthetic was not required.

Image examination

The digital camera used provided pictures in JPG format; hence graphics software (Adobe Photoshop™) was used to convert them to the standard tagged image file format (TIFF), as required by the image analysis software.

The digital picture of each flatfish was analyzed using NIH Image, a public domain image analysis program developed by the National Institute of Mental Health. NIH Image works under MacOS, with versions for both PowerPC and 68K computers, which can be downloaded as freeware from http://rsb.info.nih.gov/nih-image. Versions for PC compatible computers have been developed by Scion Corporation, running under Microsoft Windows 95/98 and Windows NT. Scion Image for Windows can be downloaded from http://www.scioncorp.com.

All distances in the images were converted to centimeters using a scaling factor. This scaling factor was obtained with an accuracy of ±1 mm from the 15 cm ruler positioned on the bottom of the white container and included in each photograph.

Fish allometric data

The digital picture of each single flatfish was analyzed using NIH Image. With the measuring tools of the program, the width, total length, and standard length values were generated (Fig. A2.1).

Fish total surface area and body surface area

To get the total surface area (fins included), the digital images were analyzed as indicated in the following sequence, starting with the black and white TIFF picture (Fig. A2.2):

a. Select 'threshold' within the 'option' menu. Increase or decrease the threshold until the fish shape, including lateral fins, edges are clearly delimited on the screen. Thresholding determines a gray intensity value that differentiates objects of interest from the background.
b. Select 'closing set' (values from 1 to 2) within 'rank filters' within the 'process' menu. This command makes the fish body denser.
c. Click on the 'options' item in the 'analyze' menu. Turn on the 'area' and 'include interior holes'

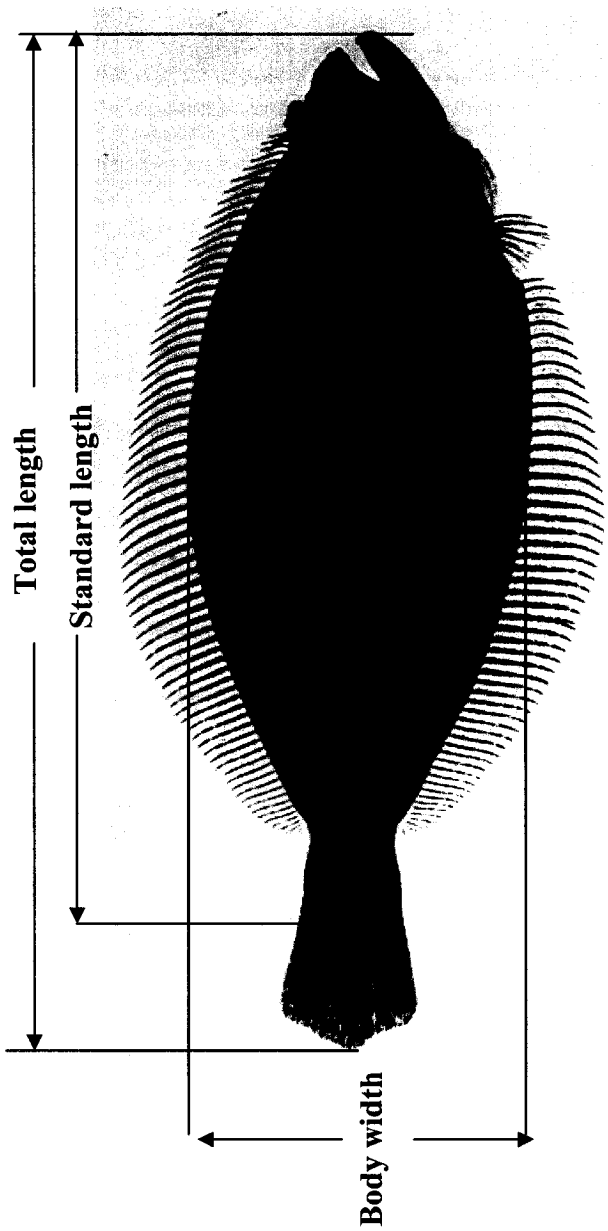

Figure A2.1.- California halibut pictures were analyzed for body width, total length, and standard length.

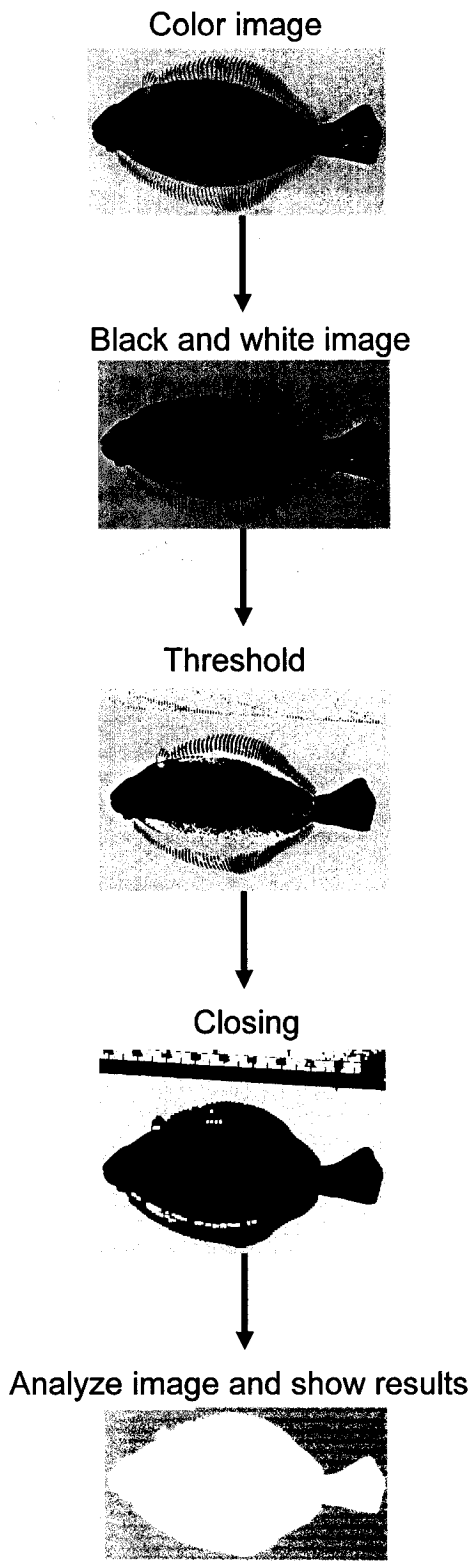

Figure A2.2.- Analysis to generate fish total surface area. See text for explanation.

d. Click on the 'analyze particles' within 'analyze' menu. Turn on the 'label particle', 'ignore particles touching edge', 'reset measurement counter', and 'label particle' options, and set the 'min particle size' to 100 and the 'max particle size' to 99999999.
e. Click on the 'show results' within the 'analyze' menu to display the measured fish area.
f. The results shown can be saved with a spreadsheet program.

To get the body surface area (fins excluded), digital images were manipulated as indicated below, starting with the black and white TIFF picture (Fig. A2.3):

a. Using the 'erase' tool, erase the caudal fin of the fish in the black and white picture. The erase action should separate the body from the tail at the fork section of the fish tail.
b. Select 'threshold' within the 'option' menu. Increase or reduce the control of the 'threshold' command until the fish body shape is outlined on the screen and the lateral fins are almost removed.
c. Select 'min erode' (choose values from 1 to 2) within 'rank filters' within the 'process' menu. This will erase the remaining fins. Sometimes some vestiges of the fins would still be attached to the fish body, and in this case the 'erase' tool should be used.
d. Select 'closing set' (choose values between 2 to 5) within 'rank filters' within the 'process' menu.

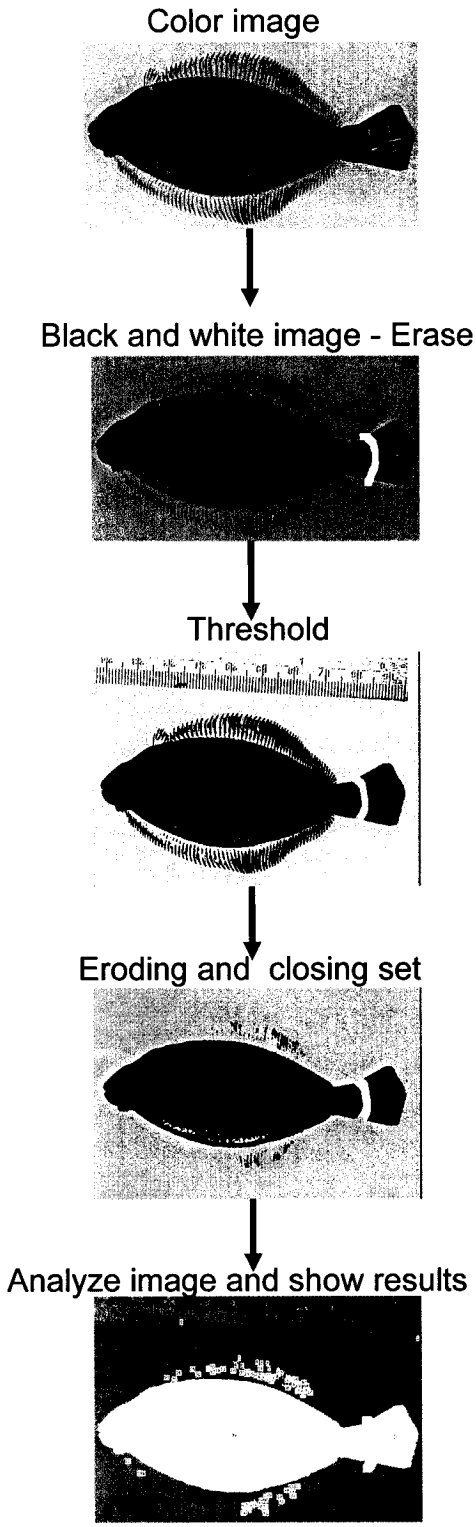

Figure A2.3.- Analysis to generate fish body surface area. See text for explanation.

e. Click on the 'options' item in the 'analyze' menu. Turn on the 'area' and 'include interior holes'
f. Click on the 'analyze particles' within the 'analyze' menu. Turn on 'label particle', 'ignore particles touching edge', 'reset measurement counter', and 'label particle' options, and set the 'min particle size' to 100 and the 'max particle size' to 99999999.
g. Click on the 'show results' within the 'analyze' menu to display the measured fish area.
h. The results shown can be saved as a file.

Management of data

The mass and allometric data can be used to establish relationships between the variables (Table A2.1). As a first approach, fish mass can be used as an independent variable, since these data are easier and quicker to measure than any of the others (Fig. A2.4 to A2.7).

Future applications of image analysis technology

With the use of image analysis technology, relationships between morphometric data and fish mass against fish TSA were established for the first time for California halibut juveniles ranging from 0.77 to 270 g.

Table A2.1.- Sample wet mass (WW) of fish and their respective allometric data (SL=standard length; TL=total length; TSA=total surface area; BSA=body surface area).

WW	SL	TL	TSA	BSA
g	cm	cm	cm^2	cm^2
1.45	4.72	5.51	8.73	5.61
2.31	5.46	6.26	12.08	7.75
1.44	4.48	5.31	8.96	5.59
1.01	4.28	4.99	7.38	4.98
2.29	5.30	6.15	11.04	6.81
1.67	4.85	5.67	9.98	6.71
1.28	4.22	5.10	8.21	5.32
1.08	4.19	5.02	7.70	4.96
51.31	14.78	16.69	81.68	56.37
40.1	13.65	15.15	73.27	51.04
46.91	14.60	16.29	80.19	56.59
48.36	15.00	17.10	83.96	59.50
35.36	14.15	15.72	70.39	49.87
42.23	14.27	16.37	88.55	53.28
44.99	14.78	16.23	76.15	52.56

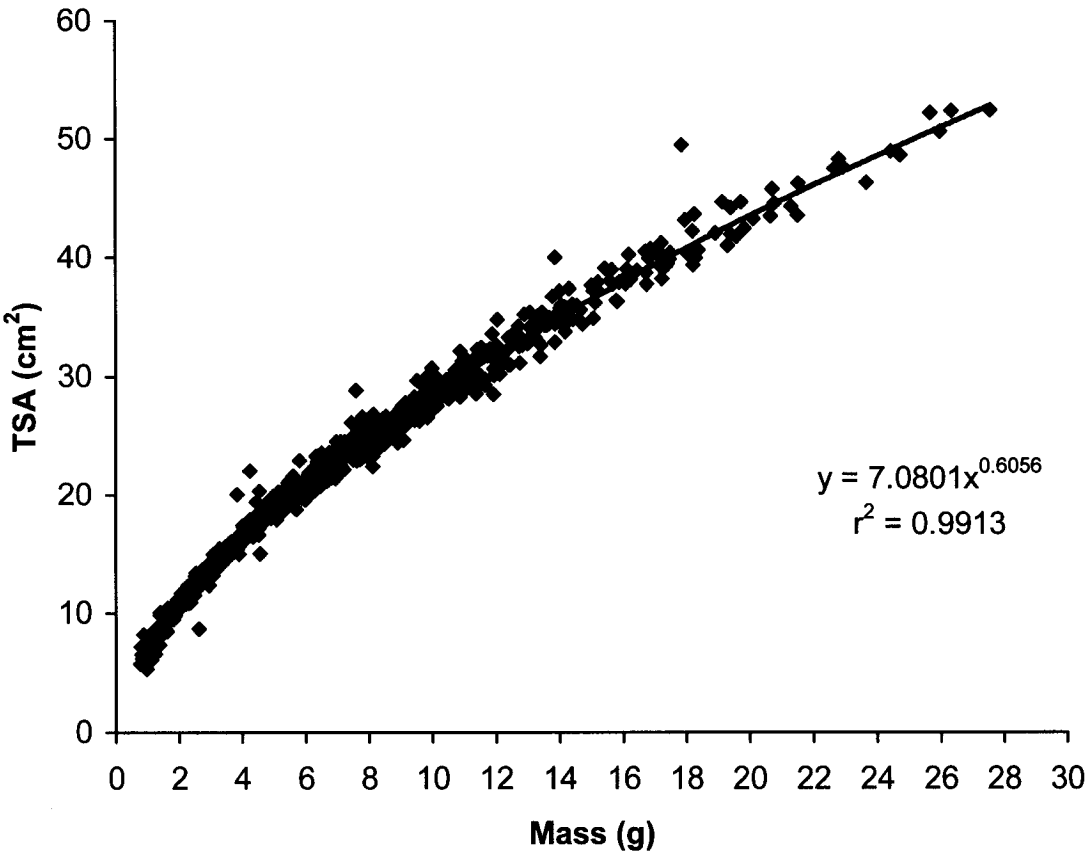

Figure A2.4.- The relationship between fish mass and fish total surface area for fish between 1.4 and 29.5 g.

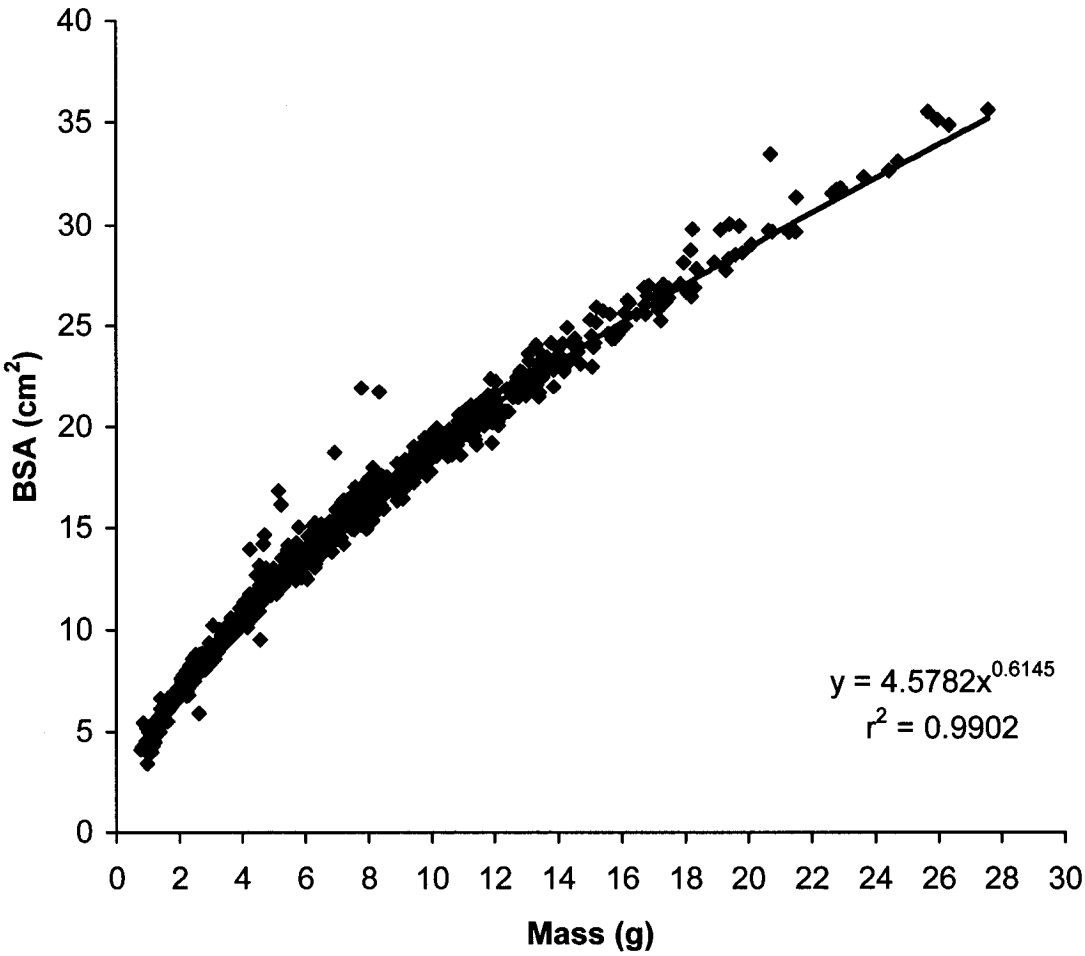

Figure A2.5.- The relationship between fish mass and fish body surface area for fish between 1.4 and 29.5 g.

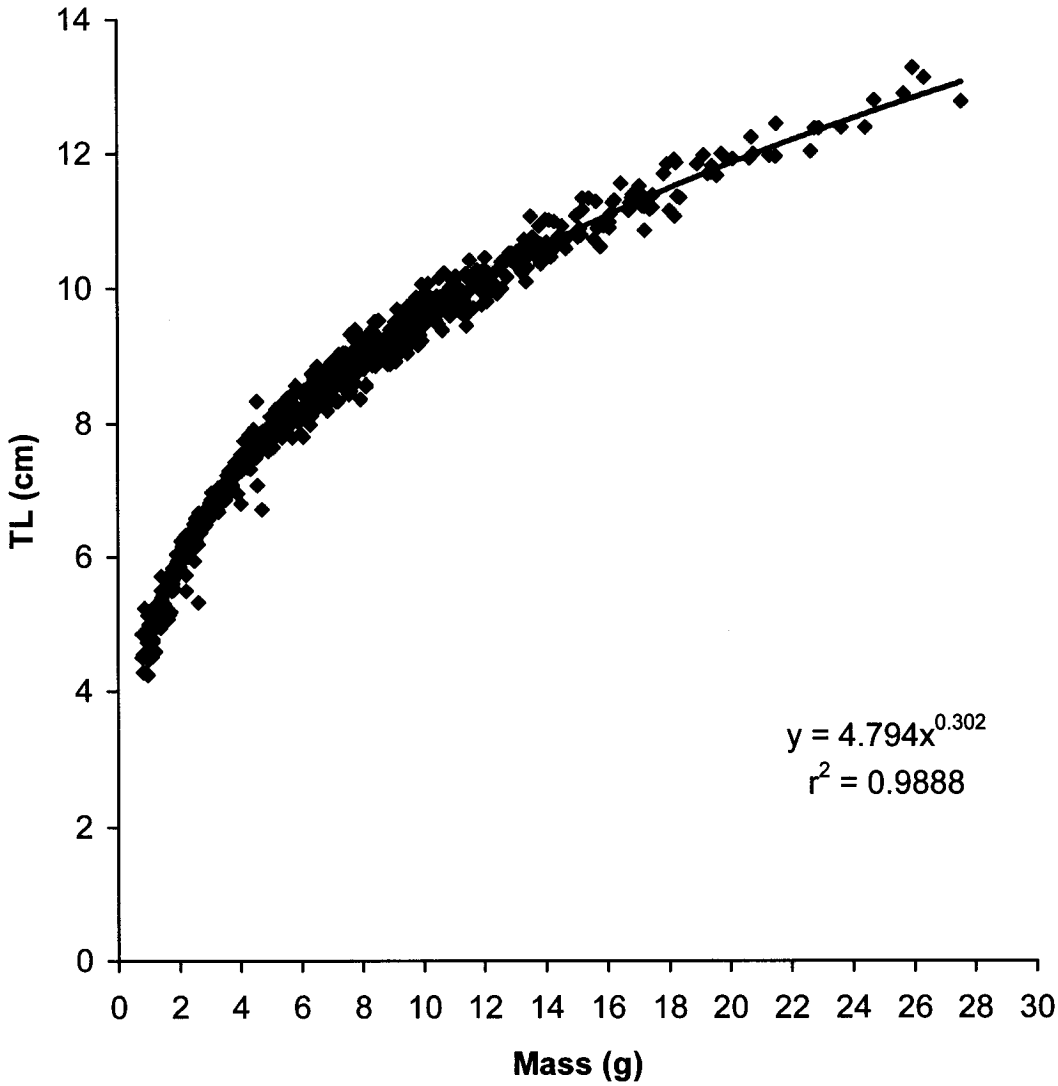

Figure A2.6.- The relationship between fish mass and fish total length for fish between 1.4 and 29.5 g.

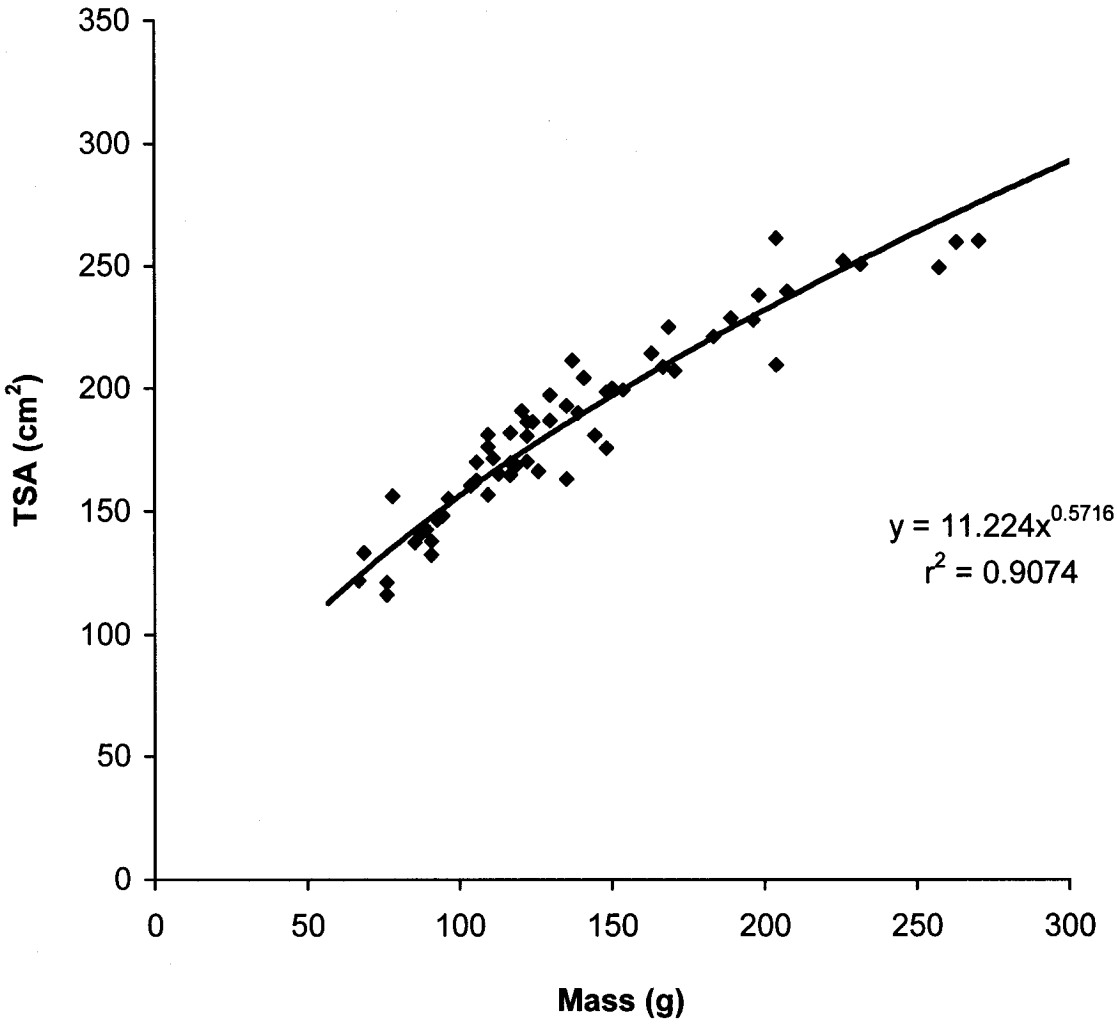

Figure A2.7.- The relationship between fish total surface area and fish mass. California halibut wet mass between 66 and 270 g.

Measurements of fish length and mass are common in aquaculture facilities. In the case of flatfish species, the surface area of the body is used for estimating of stocking density. With image analysis software, data on total length, standard length, width, and fish surface area can be obtained with minimum handling or injury of the animals. In the future it may be possible to use image analysis to measure the fish *in situ* and use the known allometric relationships to estimate fish mass (Fig. A2.4 to A2.8), without weighing and handling the fish.

After the analysis presented here was performed, it was clear to the author that the process could be automated. By first establishing a relationship between the mass of the fish and its allometric data, a sorting machine based on image analysis technology could be built. The availability of such a sorting machine would enhance the handling and management of flatfish in commercial operations, allowing for faster counting and fish mass sorting, which will reduce fish injury and will save labor.

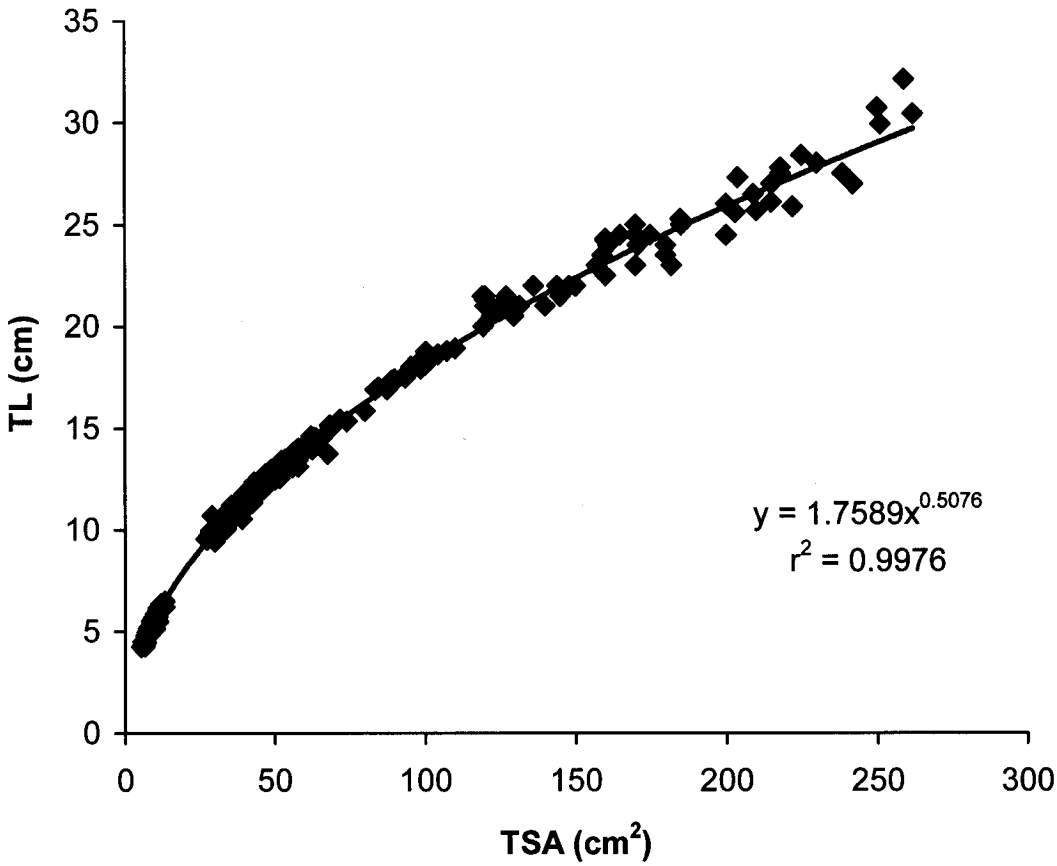

Figure A2.8.- The relationship between fish total length and fish total surface area. California halibut wet mass between 0.77 and 270 g.

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APPENDIX

A.3

UREA MEASUREMENT METHOD

INTRODUCTION

Marine animals and zooplankton excrete urea as an end product of nitrogen metabolism (Koroleff, 1983; Goeyens et al., 1998). Currently, either an indirect method based on the urease enzyme (McCarthy, 1970) or a direct diacetyl-monoxime method (Newell et al., 1967) are used for urea concentration measurements (Newell et al., 1967). A comparison of both methods was done by Price and Harrison (1987) for measuring dissolved concentration of urea in artificial seawater, who found that the direct method was easy to set up, accurate and reproducible.

The urease method, involves the enzymatic hydrolysis of urea, by urease, to carbon dioxide and ammonia. Thereafter the estimation of urea concentration can be done by measurement of ammonia content. Hence the urease method is an indirect approach to determine urea concentration. The urease method may underestimate urea concentration as a result of urease inhibition (Price and Harrison, 1987). Furthermore, urease is not specific for urea, as it is able to

catalyze the hydrolysis of at least eight other compounds producing ionized ammonia as an end product (Mulvenna and Savidge, 1992).

The diacetyl-monoxime method is a colorimetric analysis that forms a red complex between urea and thiosemicarbazide after 20 min of incubation at 85 °C (Mulvenna and Savidge, 1992). The red complex can also be observed after 72 h if the samples are left in the dark at 22 °C (Goeyens et al., 1998). Therefore the diacetyl-monoxime method is a direct estimation of the urea content of seawater.

DIACETYL MONOXIME METHOD

Based on the results of Price and Harrison (1987) it was decided to use the diacetyl-monoxime method for measuring urea-N excretion in juvenile California halibut in the present research. The procedure followed was based on the application of the diacetyl-monoxime method in clinical medicine for determining blood urea nitrogen (BUN) (http://w3.whosea.org/micro/4.htm).

PRINCIPLES OF THE METHOD

a) The urea molecule in a hot acid solution condenses with diacetyl to form a yellow product with a high absorptivity.
b) Diacetyl is an unstable molecule, and it is made as needed by acid hydrolysis of stable diacetyl monoxime.
c) Diacetyl hydrolysis occurs during the same heating step that condenses diacetyl with urea.

d) In an acidic solution the monoxime hydrolyses to give 2,3-butanedione (which is otherwise unstable).

e) Upon warming, a condensation reaction occurs with the urea in solution, which yields a five ring compound with a pink coloration.

f) Ferric ions and thiosemicarbazide are added to the solution to stabilize and intensify the pink color. The intensity of the color is proportional to the concentration of urea.

g) A comprehensive examination of the method's specificity revealed negligible interference from numerous inorganic and organic compounds (Price and Harrison, 1987). Thio-urea and bi-urea react to some extent, as do citrulline (a decomposition product of arginine) and allantoine. The common ions in natural waters do not interfere with the method (Koroleff, 1983).

h) Absorbance of the treated sample decreases very quickly with heat and light exposure after the complex formation has been completed (Goeyens et al., 1998).

Specimen collection and storage

Seawater samples should be taken directly from the water to be sampled with stoppered glass or PVC bottles. Samples should be stored at ~ 4 °C and in the dark until the analysis can be performed (preferably on the day of collection) (Koroleff, 1983).

REAGENTS

All chemicals must be analytical grade. Most of the chemicals used in this method are acids. Gloves, protective glasses, and a fume hood should be used. Care should be taken to avoid contact with skin. Instructions given in the respective MSDS for each of the reagent to be used should be followed. Appropriate safe disposal practices for each of the materials must be followed.

Stock acid reagent.

a) Dissolve 1.0 g of ferric chloride hexahydrate in 30 mL of distilled water.
b) Add 20 mL orthophosphoric acid and mix.
c) Store in a brown bottle at room temperature (25 to 35 °C). It will remain stable for 6 months.

Mixed acid reagent

a) Slowly add 100 mL of concentrated H_2SO_4 to 400 mL distilled water taken in a 1 L flat-bottom conical flask kept in an ice-cold waterbath.
b) Mix well and add 0.3 mL of stock acid reagent.
c) Mix and store in a brown bottle at room temperature (25 to 35 °C). It will remain stable for 6 months.

Stock color reagent A

a) Dissolve 2 g diacetyl monoxime in distilled water and bring the volume up to 100 mL in a volumetric flask (warm to catalyze dissolution).

b) Store in a brown bottle at room temperature (25 to 35 °C). It will remain stable for 6 months.

Stock color reagent B

a) Dissolve 0.5 g thiosemicarbazide in distilled water and bring the volume up to 100 mL in a volumetric flask. Handle this reagent carefully since is very toxic.

b) Store in a brown bottle at room temperature (25 to 35 °C). It will remain stable for 6 months.

Mixed color reagent

a) Mix 35 mL of stock color reagent A with 35 mL of stock color reagent B, and bring the volume up to 500 mL with distilled water

b) Store in a brown bottle at room temperature (25 to 35 °C). It will remain stable for 6 months.

Stock urea standard

The urea standard prepared here is different from the one suggested by the Clinical Medicine Procedure (http://w3.whosea.org/micro/4.htm). The suggested standard by the Clinical Medicine Procedure had a concentration of 10 mg urea / mL. Instead a less concentrated stock urea standard was prepared. In addition, the Clinical Medicine Procedure recommended making the stock urea standard in benzoic acid. Benzoic acid acts as a preservative to retard bacterial growth.

This method was tried, but the dissolution of urea in benzoic acid was quite difficult. Mulvenna and Savidge (1992) and Koroleff (1983) prepared the standard stock solution in distilled water and found it stable in the refrigerator for several weeks.

a) Weigh 107 mg of urea and dissolve in 100 mL of distilled water in a volumetric flask to prepare a 500 mg urea-N/L solution.
b) Store in a brown bottle in a refrigerator at 5 °C.

Working urea standard

a) Dilute 2 mL of stock urea standard in distilled water and bring volume up to 200 mL in a volumetric flask to prepare a solution of 5 mg urea-N/L.
b) Store refrigerated in a brown bottle. It will remain stable for 6 months.

Color development reagent

a) The color development reagent is prepared fresh at the time of analysis by mixing distilled water, the mixing acid reagent, and the mixed colour reagent in the ratio of 1:1:1.

CALIBRATION GRAPH OR STANDARD CURVE

A calibration graph should be constructed whenever a new set of reagents is prepared by plotting absorbance values against known prepared concentrations (standards concentrations).

Preparing standards (S) by dilution of urea stock

Standard concentrations (S) are prepared for the needed concentration by diluting aliquots from the working urea standard in distilled water (Table A3.1).

Adding color development reagent to standards

a) Prepare enough color development reagents as indicated above to analyze as many standard concentrations as have been prepared.
b) To a new set of assay tubes (triplicate for each concentration), add 2 mL of the respective standard concentration plus 8 mL of the color reagent. Agitate the tube for 5 seconds.
c) Prepare a blank sample with 2 mL of distilled water and 8 mL of the color reagent.

Heated incubation and absorbance reading

Wavelength used for absorbance measurement, incubation temperature, length of incubation, and color decay vary among the diacetyl-monoxime methods used to estimate urea concentrations in seawater (Koroleff, 1983; Price and Harrison, 1987; Mulvenna and Savidge, 1992; Goeyens et al., 1998). Preliminary tests determined that 525 nm was the wavelength that gave the highest absorbance (Fig. A3.1) and that a stable red color was reached after 80 min of incubation at 85 °C (Fig. A3.2). Color reaction decay started around minute 105 (Fig. A3.2). Incubation was carried out in a preheated Hach™ COD reactor. Absorbance was measured in a Hach™ Odissey spectrophotometer (model DR/2500).

Table A3.1.- Dilutions for standard urea solutions. All standards were prepared by diluting the amounts shown of working standard in 10 mL assay tubes (10 mm in diameter) with distilled water.

Concentration (mg Urea-N/L)	0.15 S1	0.25 S2	0.75 S3	1.00 S4
Distilled water (mL)	9.7	9.5	8.5	8
Working standard (mL)	0.3	0.5	1.5	2

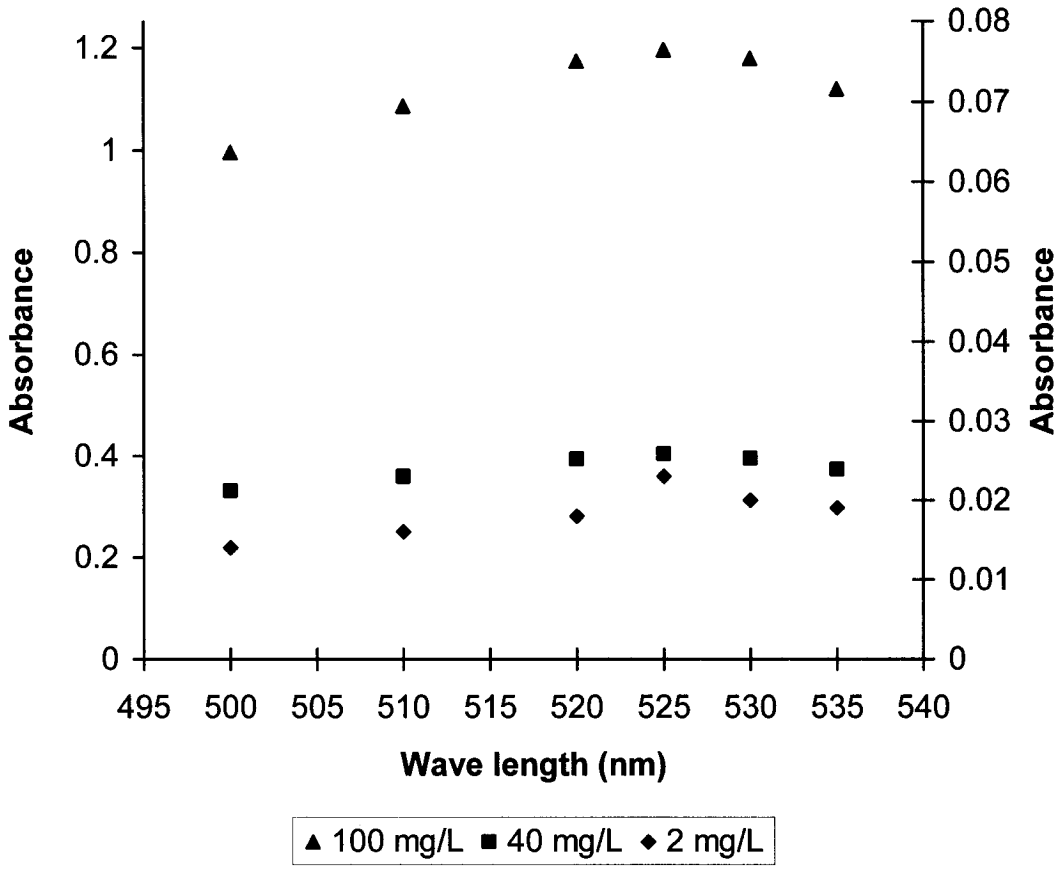

Figure A3.1.- Urea wave length absorbance. Determination of urea wave length absorbance was performed immediately after the standard concentrations were heated for 20 minutes (Mulvenna and Savidge, 1992). The 2 mg urea-N/L standard was plotted using the right y-axis. From this test, a 525 nm wave length was determined to be optimum for analysis.

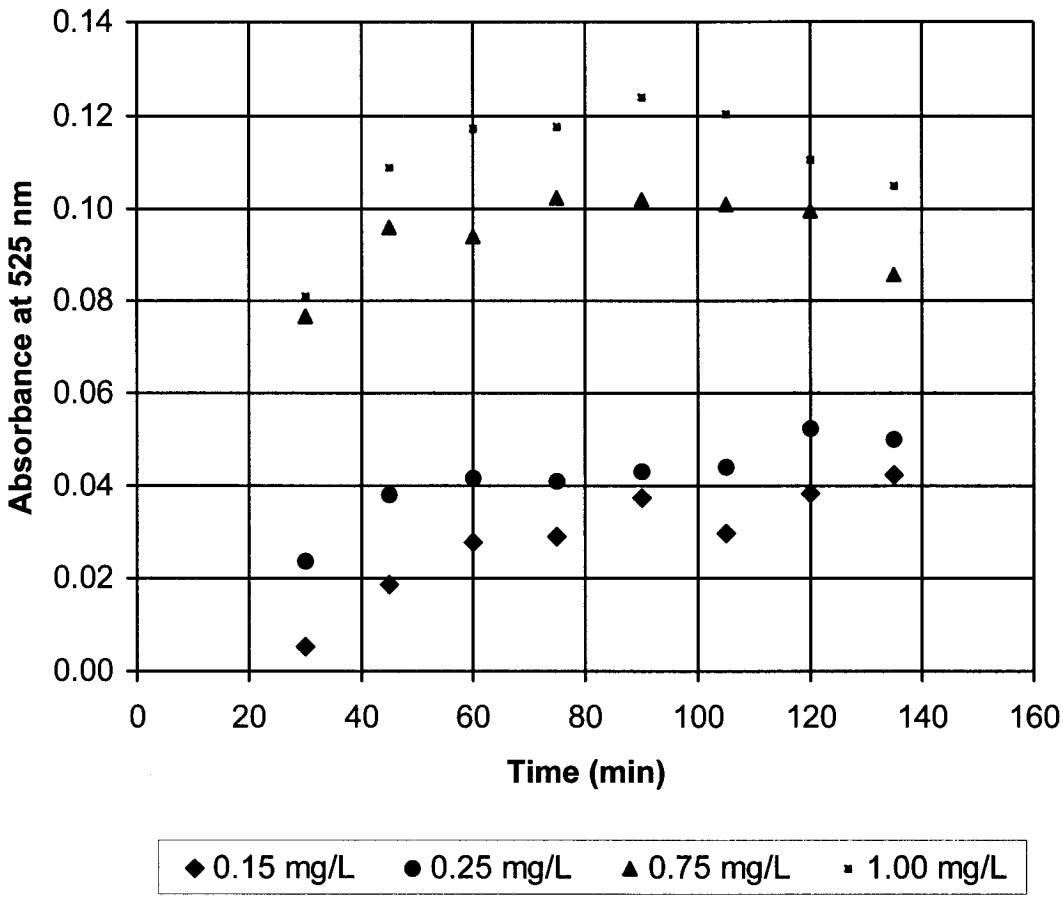

Figure A3.2.- Determination of incubation time. Four urea-N standards were prepared and heated as described in the text. Samples were analyzed for absorbance every 15 minutes while heating. It was found that a maximum absorbance was obtained at minute 80, and it remained constant up to minute ~105. After minute ~105 the color reaction started to decay at the highest concentrations and to increase at the smaller concentrations.

From the preliminary procedures, it was concluded that:

a) Once the assay tubes have been mixed, they should be incubated at 85 °C for 80 minutes.
b) Once the incubation time has expired, the absorbance at 525 nm should be measured immediately. There is no need for cooling since it was found that there is a window of 20 minutes before the reaction coloration starts to decay (Fig. A3.2). The assay tubes should be cleaned thoroughly before introducing them into the spectrophotometer chamber.
c) The spectrophotometer was set to zero with the blank between each sample to be analyzed.

Calculation and calibration graph

a) Plot the absorbance values of standard concentrations (S) against their respective concentrations.
b) Draw a regression line to relate absorbance with concentration.

ANALYSIS OF TEST SAMPLES

a) Prepare enough color development reagents as indicated above to analyze all test samples (T), plus blanks (B) and quality controls (QC).
b) A quality control is a known urea-N solution, basically a standard concentration (S) solution. It is made to check for an expected absorbance value and to check the quality of the reagents.

c) To a set of assay tubes add 2 mL of the respective test sample (T), blank, and quality control, plus 8 mL of the color reagent. Prepare three assay tubes per sample. Agitate the assay tubes for 5 seconds.

d) Incubate tubes at 85 °C for 80 minutes in the COD reactor.

e) Once the incubation time has expired, immediately read the absorbance 525 nm.

f) To determine the urea-N concentration, enter the sample absorbance into the regression line obtained from the calibration graph.

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