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ASSESSMENT OF THE REPRODUCTIVE POTENTIAL OF NEARSHORE ROCKFISH AND THE IMPACT OF ENVIRONMENTAL CONDITIONS

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<u>Final Report</u>

The overall objective of the project was to improve our understanding of the unique reproductive processes in a representative nearshore rockfish, *Sebastes rastrelliger* (grass rockfish). The research contributes to the available scientific information on the reproductive biology of an important component of the Californian nearshore fishery which may be incorporated into the development of a comprehensive management plan as envisaged in the California State legislation, Senate Bill AB 1241.

Our studies were specifically targeted at providing information that would improve our ability to make direct estimates of the contribution of individual spawns to the nearshore fishery and to develop a basis for estimating fecundity based on pre-spawn data. Study of these subjects gains considerable complexity because of the unusual reproductive strategy exhibited by rockfish. Rockfish typically exhibit a single annual reproductive cycle becoming reproductively active in late winter or spring. They are both highly fecund and viviparous. Viviparity is seen in only a small proportion of bony fish while the high levels of fecundity seen in rockfish (more than a million larvae may be extruded at each spawn depending on size and species) is more typical of oviparous teleosts. Study of developmental events in rockfish is further complicated by the lack of homology between the site of gestation in teleosts as compared with other classes of vertebrates. Oviducts (the usual site of embryonic development) do not develop in teleosts and in rockfish, gestation proceeds in the lumina of paired ovaries. Following mating and fertilization of groups of synchronously developing oocytes, embryos develop within the ovary for 1-1.5 months depending on the species. At spawning, small, 4-9 mm, larvae at an advanced stage of organogenesis are extruded to begin an extended phase of planktonic existence. The unusual character of rockfish reproduction requires a novel evaluation of events during the establishment and progression of pregnancy since regulatory mechanisms and concepts developed from more conventional fish models are not necessarily applicable directly to rockfish.

During the season of reproductive activity, rockfish release massive numbers of larvae, typically in single spawns. The potential fecundity of an individual rockfish is initially determined by the number of oocytes recruited for vitellogenesis, their successful transition through oocyte development, and fertilization success. Following fertilization, the realized reproductive output will be determined by the degree of intraovarian mortality together with larval survival during the birth process and subsequent development. We have completed a comprehensive analysis of the developmental events that culminate in the high fecundity normally expressed by rockfish. We have successfully applied biopsy techniques for the serial aspiration of ovarian tissue that

allow us to analyze the progression and construct the time course of individual events during oocyte and embryo development. By histological and quantitative morphometric analysis of sequential biopsy samples taken from individual fish (longitudinal studies), we have established a spectrum of developmental parameters that serve as convenient indices for constructing the time course of events during oocyte and embryonic development. The events chronicled include eye development and lens formation, fin-fold formation, establishment of the circulatory system and gut differentiation. By reference to these parameters, we have successfully monitored the sequence and established the timing of component phases of both oocvte and embryonic development during the establishment and progression of pregnancy. We have obtained specific information on the complete timing of embryonic development from first cleavage, through organogenesis and hatching. Our ability to successfully spawn rockfish under controlled conditions has allowed us to directly quantify realized fecundity at birth. Spawning episodes have been analyzed and the relationship between potential and realized fecundity evaluated. The length of pregnancy, estimated from the summation of the time occupied by component developmental stages, was approximately 30 days. Histological and morphometric analysis of ovarian samples, collected both crosssectionally and longitudinally, has revealed that embryos undergo extensive growth and organ development (including eve, gut and mouth formation) within the chorion with hatching occurring a few days before parturition. In spite of the extensive intra-ovarian development of the larvae, morphometric analysis of yolk volume indicated only a modest (23%) depletion of yolk reserves during major phases of organogenesis. The onset of accelerated yolk depletion was observed during the final week of gestation in association with hatching. Yolk reserves were reduced by 40% at birth and exhausted 5 days after spawning. This observation may support the view that rockfish larvae are matrotrophic during their sojourn in the ovary. A more direct evaluation of this concept might be a productive area of future research.

Our established sequence and timing of intra-ovarian events, establish a basis for determining changes in potential fecundity resulting from failure in oocyte and embryonic development and for analyzing the potential impact of environmental perturbations on reproductive success. Particular periods of oocvte and embryonic development that are susceptible to losses have been identified and quantified revealing distinct patterns of atresia. Oocyte development was characterized by extremely low levels of atresia and developmental abnormalities were not detected in the oocytes of the majority of individuals in the population studied. Significant and variable atresia (1.5-75%, depending on stage) was observed during the component phases of embryonic development and normally developing larvae could be observed adjacent to atretic larvae at an earlier stage of differentiation. Characteristically, atretic larvae contained little or no yolk. Our ability to successfully spawn rockfish under controlled conditions has also allowed us to compare the potential fecundity during intra-ovarian development with the realized fecundity at birth. Individual fish exhibiting high levels of intra-ovarian embryonic atresia released only a small proportion of abnormal larvae at spawning indicating that atretic embryos are retained and absorbed in the ovary.

In order to comprehensively address the problems currently facing the fishery, it is critical to develop an improved understanding of the environmental factors that affect sensitive developmental processes in the viviparous pattern of reproduction exhibited by rockfish that lead to a successful spawn. To this end, we have established controlled dietary regimens and environmental conditions that allow the expression of a full range of reproductive function in fish maintained for long periods in culture. Fish that previously spawned in our facility have progressed through oocyte development and vitellogenesis during the subsequent season. Also, we have observed that animals introduced into our facility in early stages of oocyte maturation become pregnant several months later in the absence of male fish indicating prolonged sperm storage in rockfish. It was consistently observed that fish with established patterns of feeding to satiation, both before and after pregnancy, did not feed while pregnant. This indicates that nutritional status prior to pregnancy rather than feeding activity following fertilization may be a primary determinant of spawn quality. These observations have important implications for the culture of the species.

Few studies have been conducted to determine the effects of environmental perturbations on the development of larvae of the genus *Sebastes*. We have performed a series of experiments in order to examine the interactive effects of salinity, temperature and nutrition during early post-spawn development in the grass rockfish. Parallel studies on the oviparous white seabass, (*Atractoscion nobilis*), have allowed us to make comparisons with the way environmental variations influence development in oviparous teleosts. These comparative studies have validated the methodology to be used in the determination of optimal environmental conditions for larval growth and development. Our data provide an insight into the unique physiological adaptations associated with viviparity that allows for high fecundity and high larval recruitment to local fisheries.

In the aggregate, our studies contribute to our understanding of the unique reproductive life-history of viviparous rockfish and the way environmental influences may impact on reproductive development to modify the full expression of normally high fecundity. This body of information is germane both to the development of a comprehensive management plan for the fishery and to the realization of the potential of rockfish for culture in food resource and replenishment programs.

Lectures presented:

2002-

Early development patterns in cultured viviparous *Sebastes rastrelliger* and oviparous *Sparus sarba* teleosts. World Aquaculture 2002, International Aquaculture Conference and Exposition of the World Aquaculture Society and China Society of Fisheries, Beijing, PRC.

Potential Fecundity and Realized Reproductive Output in Nearshore Rockfish. Sea Grant Association Fall 2002 Meeting in conjunction with California and the World Ocean '02.

2003-

Effects of Salinity on larval growth and development in oviparous white sea bass (*Atractoscion nobilus*) and viviparous teleost grass rockfish (*Sebastes rastrelliger*). Undergraduate Research Colloquium, UC Santa Barbara.

Reproductive Function in the Viviparous Rockfish, *Sebastes rastrelliger* – a candidate species for Mariculture. International UNESCO Training Course for graduate students, UNESCO Chinese Center of Marine Biotechnology, Ocean University of China, Qingdao, PRC.

Publications:

Delo LJ, Moore RK, Tsang WN, Woo NYS, Collins PM 2002 Early development patterns in cultured viviparous *Sebastes rastrelliger* and oviparous *Sparus sarba* teleosts. Proceedings of World Aquaculture 2002 Beijing, China, p 178

Manuscripts in preparation for publication:

Tsang WN, Chaille P, Collins PM 2004 Growth and developmental characteristics of cultured rockfish (Aquaculture)

Collins PM, Chaille P, Tsang WN, Moore RK 2004 Intra-ovarian embryonic development and post-spawn larval growth in the grass rockfish (*Sebastes rastrelliger*) (Biology of Reproduction)

Moore RK, Scott AP, Collins PM 2004 Morphological adaptations and functional properties of the ovary of grass rockfish (*Sebastes rastrelliger*) in relation to viviparity (Journal of Fish Biology)

Collins PM 2004 The grass rockfish (*Sebastes rastrelliger*) as a model for the endocrine regulation of viviparity (Review, Biology of Reproduction)