THE MARINE LIVE BAIT TRADE IN CALIFORNIA: A PATHWAY FOR INTRODUCTION OF NON-INDIGENOUS SPECIES?

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Committee Members:

Bruno Pernet, Ph.D. (Chair)
Christopher Lowe, Ph.D.
James Archie, Ph.D.

Department Chair:

Brian T. Livingston, Ph.D.

By Bruno Passarelli

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ABSTRACT

THE MARINE LIVE BAIT TRADE IN CALIFORNIA: A PATHWAY FOR INTRODUCTION OF NON-INDIGENOUS SPECIES?

By

Bruno Passarelli

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Several species of marine invertebrates are imported into California for use as live bait in recreational fishing. I investigated the marine live bait trade in California as a potential introduction pathway for non-indigenous species (NIS). I estimated that ~1,900,000 ghost shrimp (*Neotrypaea californiensis*), ~575,000 bloodworms (*Glycera dibranchiata*), ~600,000 pileworms *Nereis virens*), and ~1,100,000 lugworms (*Perinereis* sp.), and are imported annually into California from different parts of the world. Hitchhiker species and parasites are also commonly observed in live bait shipments along with target species. The bopyrid isopod *Ione cornuta*, a parasitic castrator, infected imported ghost shrimp at a high prevalence (14%). The short-term survival of three of these live bait NIS is not restricted by thermal conditions typically found in southern California. These results will help managers to determine the approaches that should be taken to make the live bait trade in California as environmentally and economically safe as possible.

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CHAPTER 1

INTRODUCTION

The introduction of marine non-indigenous species (NIS; species whose presence in the studied region results from anthropogenic transport) may pose a major ecological threat to coastal ecosystems and can also have negative economic consequences (Keller and Lodge, 2007; Ruiz et al., 2000). NIS that do have negative ecological or economic impacts are referred to as invasive species. From an ecological perspective, invasive species can prey upon or out-compete native species, and may also cause drastic structural modifications that may affect multiple species in the community. For example, the European green crab *Carcinus maenas* was introduced to the San Francisco Bay Area, and populations were successfully established by the late 1980s (Cohen et al., 1995). Since then, populations of two native species of clams (*Nutricola confusa* and *Nutricola tantilla*) and one native species of crab (*Hemigrapsus oregonensis*) have declined significantly due to European green crab predation (Grosholz et al., 2000). Another species of crab, the Chinese mitten crab *Eriocheir sinensis*, native to eastern Asia, was introduced in the early 1990s to the San Francisco Bay Area (Cohen and Carlton, 1997). This species caused damage in levees and stream banks as a result of their burrowing activity (Dittel and Epifanio, 2009), thus altering habitats and displacing native species. Besides ecological damage, marine invasive species can also be economically costly. For example, the wood-burrowing shipworm *Teredo navalis*, also introduced in the San

Francisco Bay Area in the early 1990s, has caused serious structural damage to boats, marinas, docks, and pilings for almost two decades, with damage estimates at about $205 million/year (Cohen and Carlton, 1995). Eradication of invasive species can be costly and impractical. Thus, a preventive approach, aiming to minimize introductions of NIS, is a more effective way to avoid the negative consequences of marine invasions (Ruesink et al., 1995).

To minimize the number of successful invasions it is clearly necessary to understand the processes involved in invasions. In this study I treat marine invasions as a three-stage process: introduction, establishment, and invasion (Figure 1). NIS are transported by human-related activities from a region where they occur naturally, the native region, to a region that is not part of their natural range, the recipient region. Introduction comprises the collection and transport of organisms, and the release of these organisms into a recipient region. Once introduced, species may or may not be able to survive and reproduce in the recipient region (i.e., become successfully established). This depends on factors such as the physiological and reproductive abilities of the introduced species as well as interactions (e.g., predation, competition, diseases) with other species present in the community. After that stage, some NIS are able to quickly spread, causing negative ecological and/or economic impacts. Spread and impact caused by a NIS is considered an invasion.

The introduction stage, including the transport of NIS and release to a recipient region, is obviously important in determining the frequency of invasions. In the past decade, numerous studies of biological invasions have shown that propagule pressure is a

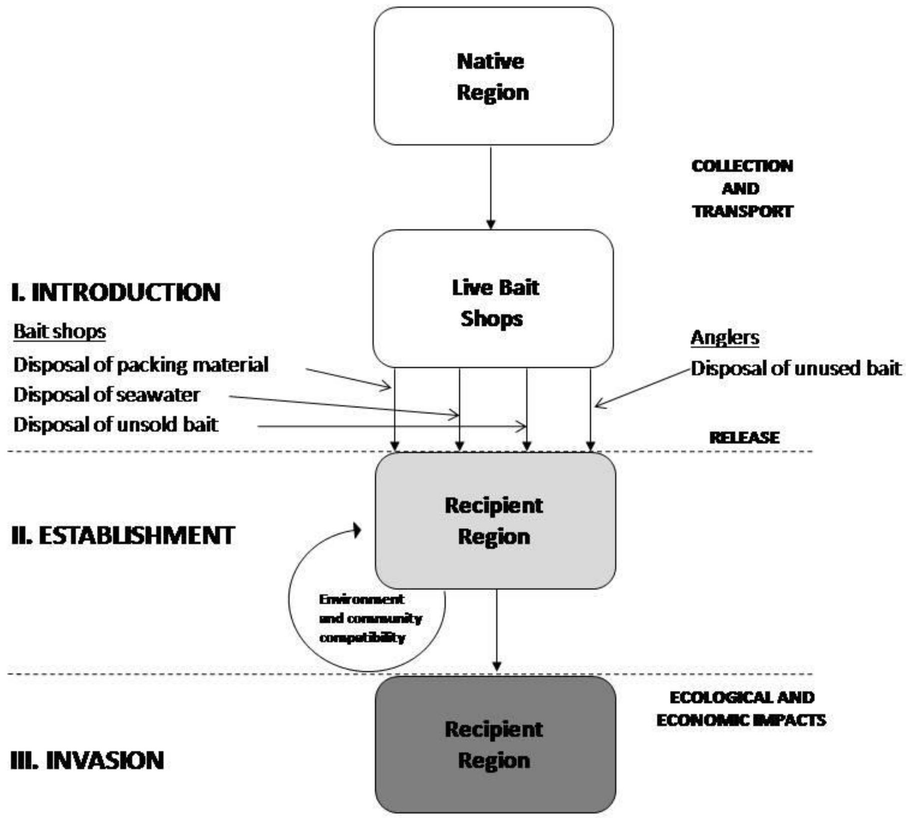

FIGURE 1. Mechanism of transfer of non-indigenous marine species of live bait from their native region to a recipient region. Species are collected and transported by distributors from their native regions to live bait shops. From bait shops, marine NIS can be introduced to the recipient region through the disposal of packaging material, seawater, unsold bait, and left-over/unused bait by anglers.

crucial parameter affecting successful establishment of NIS (Williamson, 1996; Duggan et al., 2006; Lockwood et al., 2005; Colautti et al., 2006; Leung and Mandrak, 2007; Simberloff, 2009). Propagule pressure has two components: the number of individuals introduced at any one particular release event and the number of discrete release events (Lockwood et al., 2005). Colautti et al. (2006) reviewed the recent literature characterizing invasive species or invaded habitats and found that high propagule pressure was consistently associated with successful establishment of NIS. Out of 64 studies explicitly examining propagule pressure, 55 identified a positive relationship between propagule pressure and successful establishment. Studies related to propagule pressure are strongly biased taxonomically towards plants (Colautti et al., 2006), and very few studies have addressed the effects of propagule pressure in aquatic environments (Wonham et al., 2000).

Propagules can be delivered via several pathways of introduction (Weigle et al. 2005). However, most studies of the introduction of NIS in marine systems have focused on a few major introduction pathways, specifically ballast water and hull fouling transport linked to commercial shipping (Ruiz et al., 2000). These pathways are the most important in terms of NIS diversity and numbers of individuals introduced to recipient regions. Estimates suggest that large numbers of individuals of larval and post-larval forms of several thousand species of marine organisms are transported daily around the globe in the ballast tanks of trans-oceanic vessels (Carlton and Geller, 1993).

Although ballast water and hull-fouling transfers by commercial ships have been identified as the primary mechanisms for marine introductions (Ruiz et al., 2000), several other introduction pathways may also have major consequences for coastal ecosystems (Weigle et al., 2005; Keller and Lodge, 2007). Many of these pathways are associated with the live marine species trade (e.g. seafood, aquaculture, live bait, and aquarium industries). The potential risk of introducing NIS via the live marine species trade is considered high (Weigle et al., 2005), and the trade is expected to grow by 16-24% in the next 20 years (Levine and D'Antonio, 2003). Transport of NIS via the live species trade is usually much more selective in comparison to ballast water and hull fouling transport, since this trade focuses on the transport of 'target' species. However, other organisms such as hitchhikers and parasites have been reported to occur in shipments of target species. Furthermore, the transport of marine species via the live species trade is of special concern since an effort is made to minimize mortality of the organisms, which is not the case with commercial shipping pathways. Even though the total number of individuals moved via these pathways might be low, the *per capita* rate of survival may be substantially higher for organisms transported via the live species trade in comparison to organisms transported in commercial shipping (Weigle et al., 2005).

Once transported, NIS still have to be released in the recipient region for introductions to occur. These release events may happen in a variety of ways. The *Caulerpa* invasion in California is a good example of how a NIS can be introduced through a less-studied pathway. *Caulerpa* is a type of seaweed commonly sold in the aquarium trade in California (Zaleski and Murray, 2006). *Caulerpa* infestations were detected in local populations in southern California lagoons in 2000 (Dalton 2001; Frisch and Murray, 2002). The introduction of *Caulerpa* was presumably caused by home aquarium owners disposing of this alga in these lagoons. The eradication costs for removing *Caulerpa* from those two populations reached over $4.1 million between the summers of 2000 and 2002, with additional funds being used after that (Padilla and Williams, 2004). Other examples related to the live species trade include the introduction of exotic species of fish in Florida via the aquarium trade (Semmens et al., 2004), and the introduction of the sabellid polychaete *Terebrasabella heterouncinata*, brought with imported abalone from Africa via that aquaculture trade, that resulted in an epidemic in abalone (*Haliotis* spp.) farms in California (Kuris and Culver, 1999).

While these studies have shown that the aquarium and aquaculture trades can be viable pathways for the introduction of marine NIS, other pathways have been less well studied. The introduction of marine NIS via the live bait trade, for example, has rarely been studied (Cohen et al., 2001; Weigle et al., 2005). The best data currently available are economic. Thompson and Alam (2005) estimated that over $70 million were spent in the importation of live bait into the United States (including worms and other types of bait) between 1998 and 2000. The monetary value of importation activities is useful from an economic standpoint, but can be difficult to translate into biologically relevant numbers. To assess the risks associated with the live bait trade it is fundamental that we have information on the number of organisms transported via this trade and also how many organisms get released in recipient regions.

In California, NIS of live bait are frequently used for recreational marine fishing. These include polychaete worms imported from the Northwest Atlantic Coast of the United States (‘bloodworms’, *Glycera dibranchiata*; ‘pileworms’, *Nereis virens*), Korea (‘lugworms’, *Perinereis* sp.), and Vietnam (‘nuclear worms’, *Namalycastis* sp.) (Cohen et al., 2001; Thompson and Alam, 2005). Besides these target species, hitchhikers and parasites are commonly found in the shipment of live aquatic organisms. Hitchhiker species are those that may be collected, packed, and shipped with the target species. Bait worms, for example, are commonly packed with seaweed when shipped from their place of origin, and a variety of other organisms belonging to various taxonomic groups are also found in such shipments (Carlton, 1979, 1992; Lau, 1995). In a survey of the live marine species trade in coastal Massachusetts, approximately 60% of importers reported the presence of hitchhiking species in bait shipments (Weigle et al., 2005). Although the marine live bait trade has not been extensively studied, previous surveys of NIS suggest that at least four species of marine organisms have likely been introduced into California from the East Coast of the United States packed with seaweed in baitworm shipments (Carlton and Cohen, 1998; Cohen et al., 2001).

Besides hitchhiker species found in shipments of target live bait species, parasites have been reported to infect target species of live bait. Previous studies have shown that non-indigenous species of parasitic barnacles, copepods, monogeneans, nematodes, and trematodes have become established in novel regions (Torchin et al., 2002). One of the few studies on the live bait trade in California shows that the parasitic bopyrid isopod *Ione cornuta* infects a significant percentage of the ghost shrimp *Neotrypaea californiensis* imported as live bait into California from Washington and Oregon (Pernet et al., 2008). Bopyrid isopods have the ability to castrate both male and female hosts (Munoz and George-Nascimento, 1999; Astete-Espinozal and Caceres, 2000;

McDermott, 2002) and may have drastic negative effects on the fitness of their hosts. *Ione cornuta* has been recently found infecting ghost shrimp in populations in southern California (B. Passarelli, unpublished data). Although *N. californiensis* is native to California, it is unclear if *I. cornuta* is native or non-indigenous. Previous biogeographical research shows that the distribution of *I. cornuta* ranges from British Columbia to San Francisco, California (Brusca et al., 2001). Since this species could be non-indigenous to southern California, it is important to minimize introductions to avoid negative effects to native species in local habitats.

One of the main pathways for introduction of live bait is probably the disposal of unused live bait into the aquatic environment by anglers. The chance of an individual angler introducing a NIS that will become successfully established is probably low. However, the overall probability may be very high, since there are many anglers disposing live bait in the aquatic environment (Ludwig and Leitch, 1993). No estimates are available for the marine environment, but research shows that 41% of freshwater anglers surveyed in Canada and the United States released unused live bait in freshwater systems (Litvak and Mandrak, 1993). In California, marine recreational fishing is also very common, but it is currently unclear if NIS introduced by anglers can become successfully established because we lack data on the propagule pressure of these species in local habitats. It is necessary to estimate the number of individuals transported and released (Figure 1) via the live bait trade so we can better understand the propagule pressure of NIS introduced via this pathway.

The purpose of this thesis was to begin to address some of the issues related to the introduction of NIS via the marine live bait trade in southern California. To accomplish this goal, this study focused on two specific main objectives. First, I investigated two aspects of the live bait trade that will help us have a better understanding of live bait propagule pressure and the significance of bait shops in NIS introductions in California marine environments. This included a basic description of the marine live bait trade at the bait shop level to estimate how many NIS are imported; how many individuals of each species are imported; whether they are imported seasonally; and if bait shop-mediated introductions are likely important. In addition, I assessed the possibility of importing parasites along with target bait species using one imported species, ghost shrimp and its associated parasites, as a model. Second, as a start to understanding the physiological compatibility of some of these imported NIS, I assessed their survival in relation to one physical parameter -- water temperature. Temperature is one of the most important factors determining the geographical distribution of marine organisms (Bhaud et al., 1995; Portner, 2001). If the range of temperatures found in the native region of non-indigenous live bait species is not very different from the thermal range found in southern California, then these bait species would likely survive if introduced. The results of this study will help us to begin to understand the risks of marine invasions associated with the live bait trade in California.

CHAPTER 2

MATERIALS AND METHODS

Transport and Release of Bait Species: Investigating the Numbers and Release Pathways of NIS Imported into California via the Marine Live Bait Trade

The main goal of this component of the study was to estimate the number of organisms imported into California via the marine live bait trade so we can begin to understand the propagule pressure of NIS introduced via this pathway. Other aspects of the live bait trade in California such as the origin of imported bait, seasonality in importation, presence and disposal of packing materials and hitchhiker species, and disposal of unsold live bait were also investigated to help determine the importance of bait shops in mediating the introduction of NIS.

The data in this study were collected from surveys of bait shops selling marine live bait in California. These shops were identified in a three stage process. First, I used two online directories to compile a list of businesses in California that were likely to sell live bait. I combined businesses listed on *www.baitnet.com*, a website with a large database of bait shops and fishing tackle shops in the United States that has been used as a source in other biological invasion studies (Pico and Collins, 2008) with businesses listed under "Fishing Bait and Tackle" in the state of California on the website *www.superpages.com/yellowpages*. A total of 834 businesses were included in this initial stage.

Second, businesses were categorized by county. Businesses found in counties with marine shorelines were selected for further analysis since they are more likely to sell marine organisms as live bait in comparison to businesses in non-coastal counties that presumably provide primarily non-marine live bait (e.g., night crawlers) for fishing in freshwater. I verified this assumption by calling ten bait shops located in non-coastal counties and inquiring if they sold marine live bait; none of these shops sold marine live bait. However, the Sacramento River delta region was an exception since marine live bait organisms such as ghost shrimp are commonly used for sturgeon recreational fishing in fresh water systems in that area. Although there is presumably no risk of introducing marine live bait species in those areas since salinity levels are likely too low for marine species to survive, businesses located in counties in the Sacramento River delta region were also included in the list of survey candidates in order to estimate the total number of marine live bait organisms imported into California annually. A total of 444 businesses, 404 located in coastal counties and 40 in the Sacramento delta region, were identified as candidates for a preliminary telephone survey.

Third, a preliminary telephone survey of the 444 businesses found in coastal counties and the Sacramento delta region was conducted in September 2008 to identify which of those sold marine organisms as live bait. A total of 64 bait shops selling marine live bait were found in this process.

I conducted a more detailed mail survey with those 64 bait shops. The survey consisted of 12 questions (Appendix A) designed to assess the quantity and other aspects (e.g. origin, seasonal patterns, packing materials, mode of disposal of unsold bait) of commonly sold marine live bait organisms in California. In February 2009, I mailed surveys to the 64 bait shops with a signed cover letter (Appendix B) and a pre-stamped, addressed return envelope. Businesses were contacted by telephone 10-14 days after the questionnaires were mailed to encourage return of questionnaires. As an attempt to increase the number of survey participants, I revised the bait shop database in August 2009 using the same methods and found six new bait shops that were not listed when the original database was compiled. I mailed surveys to the six newly identified bait shops, and also mailed surveys one more time to bait shops that did not respond to the first attempt. In total, the survey was mailed to 70 bait shops.

Data from returned surveys were used to describe aspects of the marine live bait trade in California. To investigate the transport of NIS into California via the live bait trade (Figure 1) I estimated the number of individuals of target species of marine live bait imported into California annually. The number of individuals imported per bait shop was estimated in the following manner. First, I calculated the potential number of bait shops selling each marine live bait species in California. This was calculated by multiplying the percentage of survey respondents selling a particular species of live bait by the total number of bait shops (70) identified as selling marine live bait. For example, if 46% of survey respondents reported selling ghost shrimp that percentage (0.46) was multiplied by 70 for an estimate of 32 potential bait shops selling ghost shrimp in the state. Next, I calculated the average number of individuals imported annually per shop by adding the numbers of imported individuals shrimp reported by each survey respondent and dividing it by the number of survey respondents. For example, if the total number of ghost shrimp imported by bait shops was 650,804 and eleven shops provided those numbers, then 650,804 was divided by 11 for an estimate of 59,164 ghost shrimp imported annually per shop. Finally, I multiplied the average number of each particular species imported annually per shop by the potential number of bait shops selling this species to estimate the number of individuals imported annually into California. In the above example, 59,164 ghost shrimp imported per shop multiplied by 32 potential shops provided an estimate of 1,893,248 ghost shrimp imported annually into California.

Seasonality of live bait sales was determined for each target species of live bait by calculating the percentage of shops indicating high season for each month of the year. The native region, sales trend, and the year when target species were first imported to California are reported according to survey responses. Hitchhikers and type of packing material used are described for each the target species' trades. To better understand the release of NIS into local habitats (Figure 1) I also reported the methods of disposal of packing materials, seawater used to keep NIS, and unsold bait for each of the target species' trades directly from responses obtained from the survey questionnaire.

Transport of Parasites: Estimating the Quantities of Parasites Imported into California via Ghost Shrimp Trade

To assess the possibility of importing parasites along with target bait species, I investigated the ghost shrimp trade in California. This trade was used as a model for three reasons. First, the ghost shrimp *Neotrypaea californiensis* is commonly found for sale in bait shops in California. Ghost shrimp are native to California, but specimens sold in bait shops in California are imported from Oregon and Washington. Second, ghost shrimp may be infected with at least three types of parasites (bopyrid isopods, cestode tapeworms, and nematodes). Third, at least one of the parasites infecting ghost shrimp, the bopyrid *Ione cornuta*, may be non-indigenous to southern California and may negatively affect local populations of ghost shrimp.

To determine the number of parasites imported into California via the ghost shrimp trade I purchased and inspected 100 ghost shrimp (imported from Oregon and Washington) quarterly between June 2008 and January 2010 from two bait shops in southern California. For each ghost shrimp, I determined carapace length (mm), sex (based on the presence [females] or absence [males] of pleopods on the second pleomere), reproductive status of females (brooding vs. non-brooding), and prevalence of three types of parasites: bopyrid isopods, nematodes, and trypanorynch cestodes. Ghost shrimp were measured, sexed, and inspected for parasites within 24 hours after purchase. Bopyrid isopods were detected by inspecting the gill chamber of ghost shrimp. Nematodes and trypanorhynchs were detected by removing the cardiac stomach and digestive glands, respectively, pressing them between two microscope slides, and inspecting with a dissecting microscope. Parasite prevalence for each of the three types of parasites was determined by dividing the total number of infected ghost shrimp by the total number of ghost shrimp inspected x 100. Clopper-Pearson 95% confidence intervals for parasite prevalence were determined according to Bush et al. (1997).

In addition, I conducted field surveys to determine if parasites infecting imported ghost shrimp are non-indigenous or native to southern California. The natural ranges of these parasites are not well known. My assumption was that if parasites were not found in southern California populations they may be non-indigenous. Between June 2008 and January 2010, I collected ghost shrimp in 13 mudflats along the west coast of the United States. Point Conception is a site associated with biogeographic boundaries for many species of marine invertebrates on the coast of the eastern Pacific (Dawson, 2001). Since the ghost shrimp trade moves individuals from northern sites to bait shops located south of Pt. Conception, it is possible that parasites native to northern sites are transported to southern sites, where they may be non-indigenous, along with ghost shrimp hosts. Ghost shrimp populations were sampled at four sites south of Point Conception (all in southern California) and nine sites north of Point Conception (three in central California, three in Oregon, and three in Washington) (Figure 2). I used an Alvey bait pump to collect 100 ghost shrimp during low tide cycles in each of the locations. Whenever it was not possible to collect 100 ghost shrimp I collected and inspected as many specimens as I was able to find. Parasite prevalence for each site and collection date of the three types of parasites was determined in the same manner as for imported ghost shrimp purchased from bait shops.

Two co-occurring species of ghost shrimp, *Neotrypaea californiensis* and *N. gigas*, were found in some of the collection sites in this study. During most of this study, I did not know how to differentiate these two species in the field, with the exception of adult males that were differentiated due to characters in their major claw. However, a

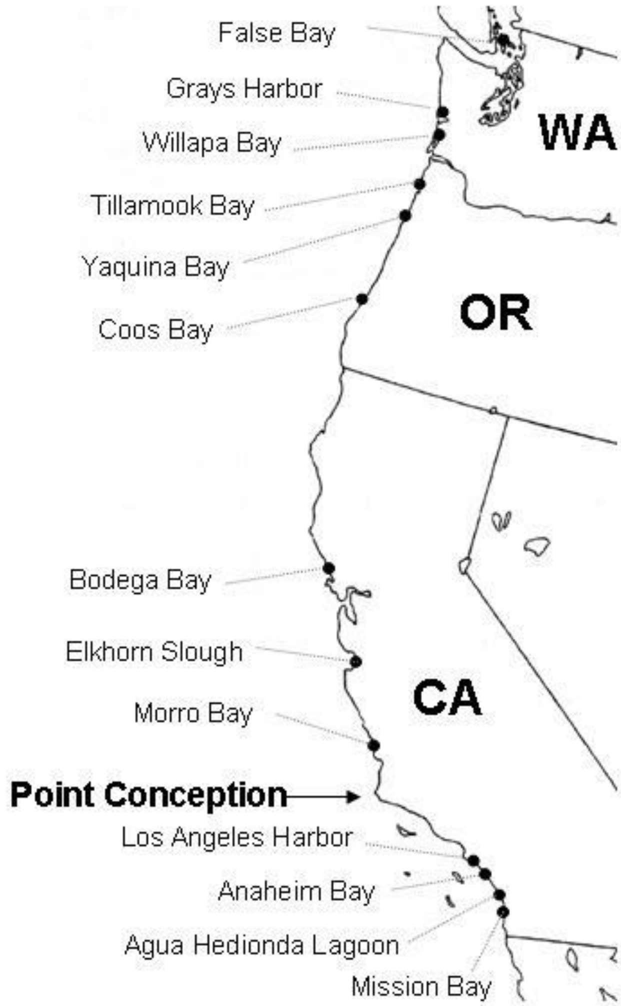

FIGURE 2. Map of ghost shrimp collection sites on the west coast of the United States.

recent study shows that females and juveniles can be distinguished morphologically by differences in the length and shape of their eyestalks (Pernet et al. 2010). In this study, specimens were identified in the laboratory as either *N. californiensis* or *N. gigas* by distinguishing the major claw of adult males throughout the study, and eyestalks of all individuals from September 2009 and on. Southern California collections were conducted quarterly between June 2008 and December 2009. Central California, Oregon, and Washington sites were sampled in two summers (June 2008 and June 2009) and two winters (Jan 2009 and Dec 2009/Jan 2010).

Fisher's exact tests were performed using the software Quantitative Parasitology 3.0 to compare parasite prevalence according to site (north versus south of Pt. Conception and bait shops versus southern California), sex (males versus females), reproductive status of females (brooding versus non-brooding), and season (summer versus winter) according to Rozsa et al. (2000).

Establishment: Survival of NIS Imported via the Live Bait Trade in Relation to Southern California Temperatures

To assess if species transported via the live bait trade have the potential of becoming established in local marine habitats I addressed the following question: Can NIS of live bait survive when exposed to temperatures typical of southern California coastal habitats? I evaluated this question by exposing imported NIS of bait for a short period of time to temperatures found in southern California. Temperature tolerance experiments were carried out at Cabrillo Marine Aquarium in San Pedro, California, between July and September 2009.

The experimental set-up consisted of three water baths holding 24 1L plastic containers filled with aerated 33-ppt filtered seawater obtained from a well on outer Cabrillo Beach, San Pedro, California. The temperatures used in the three treatments in this study were chosen based on the thermal range found locally, including shallow water habitats. Temperatures in southern California coastal waters typically range between 12.7- 21.1^{o}C (http://www.nodc.noaa.gov/dsdt/cwtg/spac.html), with an average of approximately 16^{o}C. In fact, many public aquariums keep tanks holding local species of algae, fishes, and invertebrates at 16^{o}C (J. Landesman, pers. comm.). However, the temperature range in shallow water habitats can be slightly greater than the range found in coastal waters (T. Farrugia, California State University Long Beach, pers. comm.). In this study we set up water baths with chillers or heaters at temperatures close to low (~12^{o}C), intermediate (~16^{o}C), and high (~24^{o}C) temperatures typically found in southern California marine coastal habitats. I insulated all water baths with insulation foam (2.5-3.8 cm) to minimize temperature fluctuations caused by variation in external temperature. Temperature was measured at 30 minute intervals throughout the course of the experiment using HOBO UA-002-08 pendant data loggers placed at the bottom of each of the bath tanks (Table 1).

Four species of non-indigenous baitworms are commonly sold in bait shops in California: bloodworms (*Glycera dibranchiata*), pileworms (*Nereis virens*), lugworms (*Perinereis* sp.), and nuclear worms (*Namalycastis* sp.). Three of those (*G. dibranchiata*, *Perinereis* sp., and *Namalycastis* sp.) are found for sale in southern California. *Namalycastis* sp., sold as ‘nuclear worms’, were not used in this experiment because they

TABLE 1. Mean Temperature (°C) ± SE in the Three Experimental Treatments Over the Course of the Experiment

Temperature	Run 1	Run 2	Run 3	Run 4	Run 5
Low	12.46 ± 0.024	12.47 ± 0.024	12.47 ± 0.026	12.23 ± 0.033	9.85 ± 0.057
Intermediate	15.67 ± 0.021	15.73 ± 0.018	15.67 ± 0.020	15.75 ± 0.038	15.53 ± 0.024
High	24.95 ± 0.028	23.51 ± 0.046	22.93 ± 0.150	24.38 ± 0.046	23.62 ± 0.082

are not common in bait shops in southern California, and also because of their high retail price. I tested the short-term survival of *G. dibranchiata* and *Perinereis* sp. in relation to temperature in this experiment. Besides the two species of baitworms, I also used *Ione cornuta*, the parasitic castrator isopod found in imported ghost shrimp, to test if parasites imported with live bait can survive in local temperature conditions. Specimens were purchased at two bait shops near Long Beach, California. All *G. dibranchiata* and *I. cornuta* were purchase from one of the bait shops and all *Perinereis* sp. were purchased at the other bait shop. I randomly distributed live specimens of *G. dibranchiata*, *Perinereis* sp., and *I. cornuta* individually into containers in the three water baths (Figure 3). *Ione cornuta* infecting ghost shrimp were removed from their hosts prior to the experiment. Animals were exposed to experimental conditions within two hours after purchase. Specimens were not acclimated before they were exposed to experimental conditions because I aimed to simulate disposal of leftover bait into marine habitats. Specimens were exposed to treatment temperatures for five days and were not fed over the course of the study. Partial water changes (100-200 ml) were performed daily using filtered seawater at treatment temperature.

I assessed survival, as indicated by movement, at twenty four-hours intervals throughout the experiment. The experiment was replicated five times with a total of forty observations for each bait species at the three temperature treatments. The data were pooled for each species across the five runs, and survival at day five was compared for each species separately using one-way ANOVA at the $p < 0.05$ level. Data for all species were

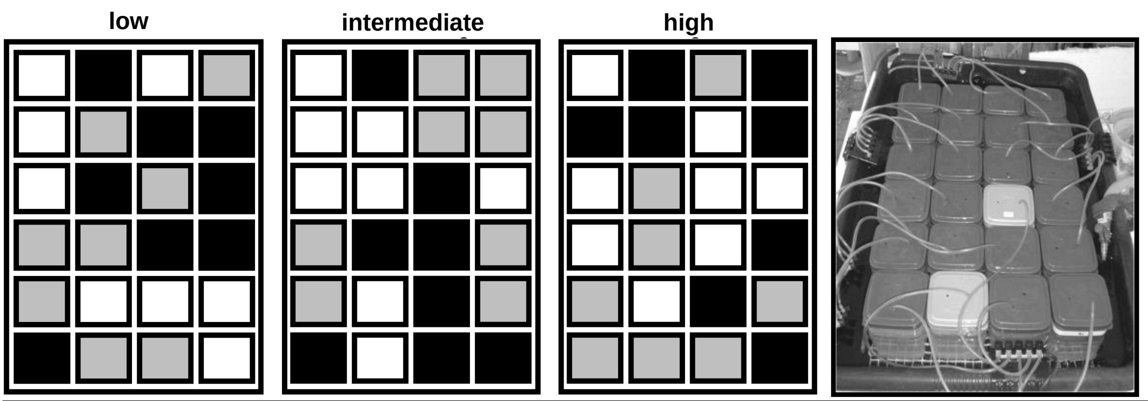

FIGURE 3. Overview of the experimental set-up. Eight individuals of *Glycera dibranchiata* (white), *Perinereis* sp. (black) and *Ione cornuta* (gray) were randomly distributed in each of the three thermal water baths. Picture of one of the thermal water baths is shown on the right.

normally distributed with homogeneous variances. Statistics were calculated using GraphPad Prism software.

CHAPTER 3

RESULTS

<u>Transport and Release of Bait Species: Investigating the Numbers and Release Pathways of NIS Imported into California via the Marine Live Bait Trade</u>

A total of 70 bait shops selling marine live bait were found in coastal and Sacramento Delta counties. The geographic distribution of bait shops selling marine live bait in California is uneven, with almost 90% of the shops found either in the San Francisco Bay Area/Sacramento Delta Region (~57%), or in metropolitan southern California (~31%). The distribution by county of bait shops selling marine live bait in California is shown in Figure 4.

A total of twenty four surveys were returned from the 70 bait shops selling marine live bait in California. Eight additional surveys were returned as undeliverable. The total return rate was 38.7% (24 out of 70) of the deliverable surveys. On average, each responding bait shop carried 2.5 species of marine live bait. The most commonly sold species of marine live bait in California were pileworms, bloodworms, and ghost shrimp with 62.5%, 50%, and 46% of survey respondents reportedly selling these species, respectively. Lugworms and nuclear worms were both reportedly sold at 17% of the bait shops responding to the survey. All of the bait shops reported that these species are imported into California from other states or countries (Table 2). According to survey results, baitworms have been imported into California at least since the early 1970's,

FIGURE 4. Geographic distribution by county of bait shops selling marine live bait and of live bait species sold in California. Number of bait shops selling marine live bait is in parentheses. Numbers after parentheses indicate the species sold in that particular county according to mail survey responses. Not all species were sold at every shop in each county.

TABLE 2. Origin and Date of Beginning of Importation of Marine Species of Live Bait in California According to Data Obtained from Mail Survey Responses

Live bait species	Species name	Origin	Sold in California at least since
Bloodworms	*Glycera dibranchiata*	Maine	1972
Ghost shrimp	*Neotrypaea californiensis*	Oregon, Washington	1978
Lugworms	*Perinereis* sp.	South Korea	1972
Nuclear worms	*Namalycastis* sp.	Vietnam	1994
Pileworms	*Nereis virens*	Maine, Massachussets	1972

while ghost shrimp have been imported at least since the late 1970's. Two bait shops reportedly imported other non-marine types of bait (minnows and mud-suckers) from out-of-state. Information about origin and date of beginning of importation for individual target species of marine live bait is reported in Table 2. Approximately one third of survey respondents reported selling "grass shrimp." I tentatively identified this shrimp as the California bay shrimp, *Crangon franciscorum*, one of the most common native species of shrimp in central California. All bait shops indicated California as the origin of this shrimp; therefore, it is unlikely that this particular live bait species is a NIS.

To estimate the number of individuals of each species imported into California annually, I multiplied the average number of individuals imported annually per responding bait shop for each species and multiplied that number by the potential number of bait shops selling that particular species (Table 3). Bootstrapping confidence intervals (BC percentile method) were calculated for the average number of individuals imported annually per bait shop using 1,000 bootstrap replicates in Minitab. My estimates suggest that almost 1,900,000 ghost shrimp are imported annually into California. Among baitworm species, the two species from the Northeast coast of the United States are commonly imported, with approximately 600,000 pileworms and nearly 575,000 bloodworms stocked in California bait shops annually. Over one million lugworms were estimated to be imported annually from Korea, and a few more than 700 nuclear worms are likely imported from Vietnam. Seasonality of sales of marine bait species in California varied according to the type of bait. Survey responses suggest that there are different seasonal patterns for baitworms and bait shrimp (Figure 5). Baitworm species

TABLE 3. Estimated Number of Individuals of Five Species Imported as Marine Live Bait into California Annually

Marine live bait species	A Number of survey respondents (out of 24)	B Percentage of survey respondents (out of 24)	C Potential number of bait shops (out of 70)	D Average number stocked per shop annually (number of respondents providing estimate) {95% C.I.}	E Estimated number of individuals imported into CA annually {95% C.I.}
Glycera dibranchiata (bloodworms)	12	50	35	16,408 (8) {9,095 ; 25,720}	574,280 {318,325 ; 900,200}
Perinereis sp. (lugworms)	4	17	12	93,000 (1) {n/a}	1,116,000 {n/a}
Namalycastis sp. (nuclear worms)	4	17	12	60 (1) {n/a}	720 {n/a}
Nereis virens (pileworms)	15	62.5	44	13,635 (8) {5,175 ; 25,670}	599,940 {227,700 ; 1,129,480}
Neotrypaea californiensis (ghost shrimp)	11	46	32	59,164 (7) {14,700 ; 118,714}	1,893,248 {470,400 ; 3,798,848}

A - Number of survey respondents selling this species of live bait.
B - Percentage of survey respondents selling this species of live bait.
C - Potential number of bait shops selling this species in California (column B x 70)/100.
D - Average number of individuals stocked per shop annually calculated from survey data. Number in parenthesis indicates survey respondents providing data on number of individuals stocked annually (question 5 in Appendix A).
E - State estimate of the number of individuals imported annually (column C x column D).

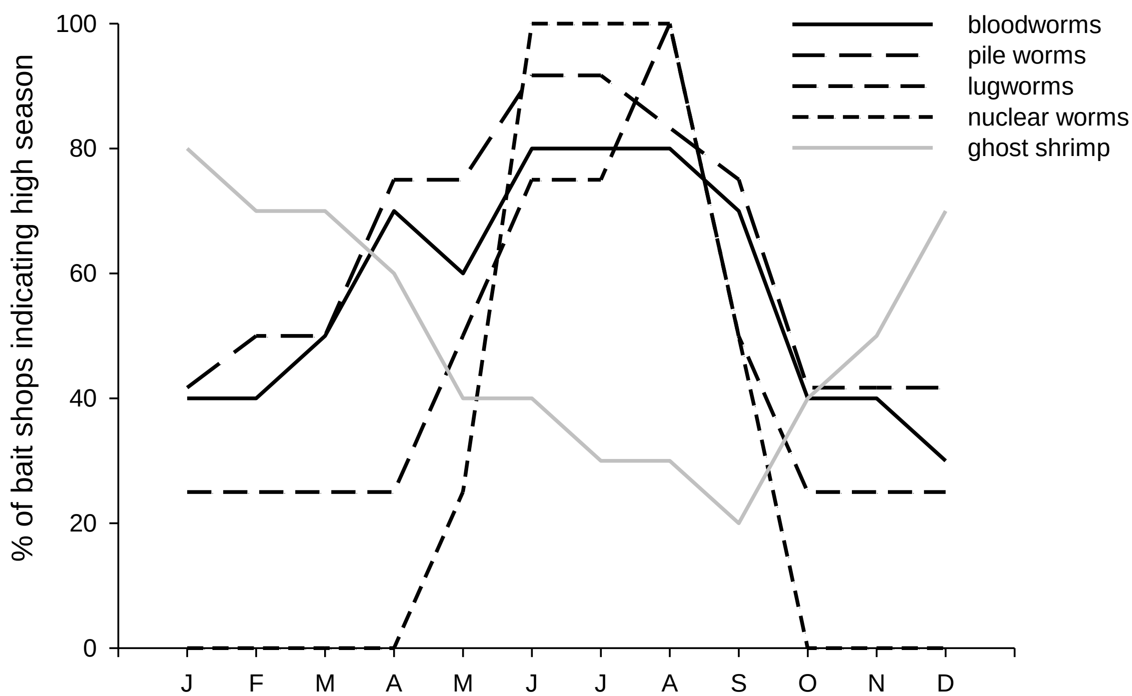

FIGURE 5. Sales seasonality of marine live bait in California. Overall, baitworm sales peak in the summer while ghost shrimp are sold the most during winter months.

showed a peak in sales during the summer while ghost shrimp had a peak in sales during winter months. For nearly all species, any given month of the year was reported by at least one bait shop as the time of the year when sales are the highest. The only exception to this was nuclear worms. No bait shop reported high sales for nuclear worms between November and March.

Most survey respondents reported seaweeds as the main packing material in live bait shipping, but seawater packing was also common (Figure 6a). Hitchhiker species were reportedly present in shipments of nearly all target species of live bait, the exception being lugworms (Figure 6b). Survey respondents noticed the presence of several organisms either in the packing materials or on the bait itself. These included clams, snails, barnacles, worms, crabs, shrimp, sea jellies, finfish, sand fleas, and seaweeds. Different methods were used for disposing of packing materials and seawater used to keep live bait. Bait shops commonly reported giving seaweeds found in shipments to their customers along with live bait, but other methods of disposal were also used. Live bait shops disposed of unsold live bait by throwing it in the trash, toilet, or other method of disposal, but a few shops also reported to give unsold live bait to their customers (Table 4).

Transport of Parasites: Estimating the Quantities of Parasites Imported into California via Ghost Shrimp Trade

To determine what parasites are imported to California via the ghost shrimp trade and if these parasites are native to California, I inspected ghost shrimp purchased from bait shops and collected from field populations for parasites. Between June 2008 and January 2010, I sampled a total of 7,336 ghost shrimp in 77 sampling events including

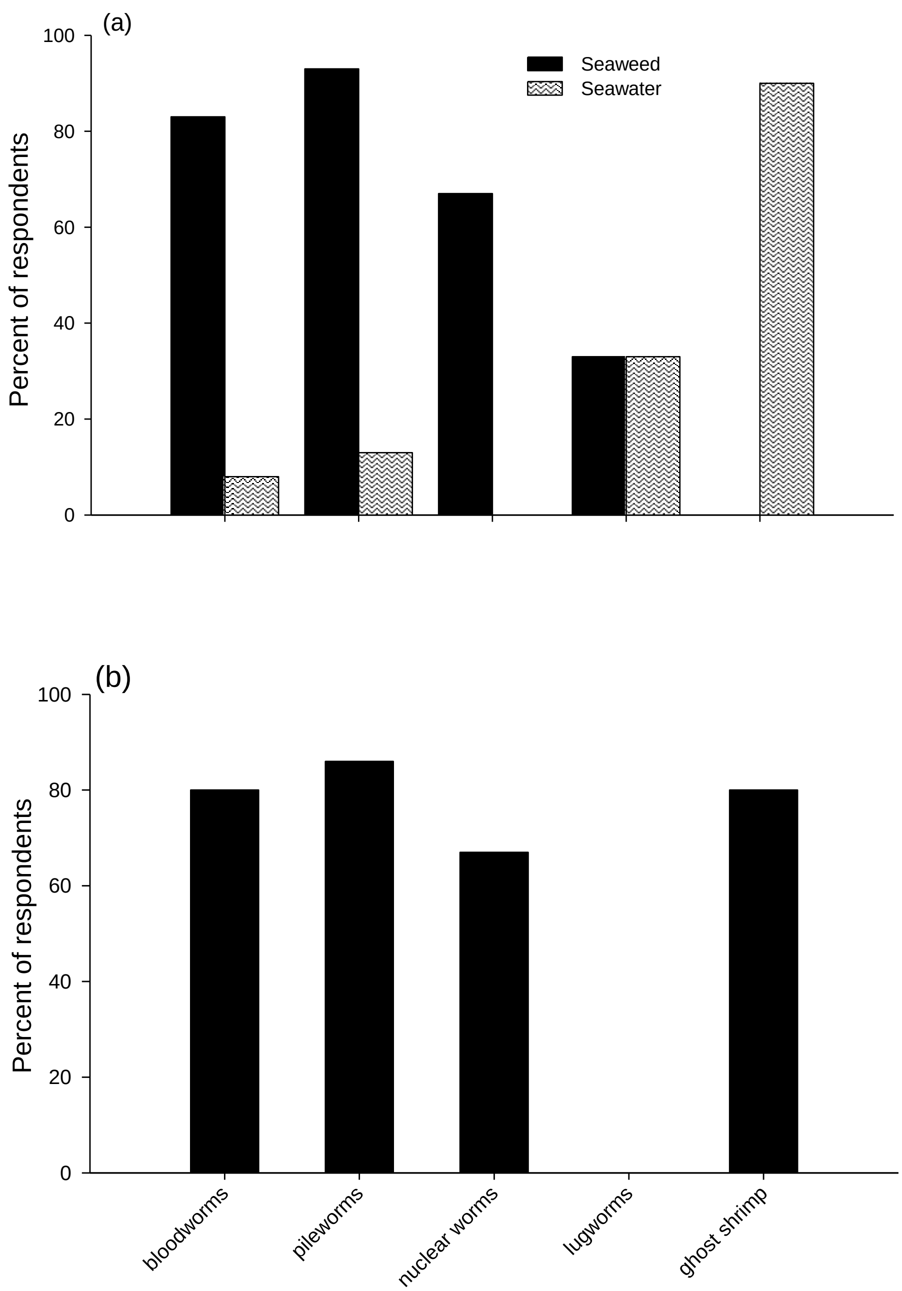

FIGURE 6. Percentage of respondents importing marine NIS shipped in seaweed or seawater packing material (a) and observing hitchhikers in shipments of target bait species (b).

TABLE 4. Methods of Disposal of Seaweed, Seawater, and Unsold Live Bait Reported in Live Bait Survey

Live bait species	N	Disposal of packing materials	Disposal of sea water	Disposal of unsold bait
bloodworms	12	give it to customers (9)	do not use SW (6)	give it to customers (0)
		ocean or bay (1)	ocean or bay (0)	return to dealer (0)
		sink (0)	sink (1)	sell all (1)
		storm drain (1)	storm drain (1)	trash (10)
		trash (7)	toilet (0)	other* (1)
		no answer (0)	no answer (5)	no answer (0)
pileworms	15	give it to customers (11)	do not use SW (7)	give it to customers (1)
		ocean or bay (1)	ocean or bay (1)	return to dealer (1)
		sink (1)	sink (2)	sell all (2)
		storm drain (1)	storm drain (1)	trash (7)
		trash (7)	toilet (0)	other* (1)
		no answer (0)	no answer (4)	no answer (2)
lugworms	3	give it to customers (1)	do not use SW (0)	give it to customers (0)
		ocean or bay (0)	ocean or bay (0)	return to dealer (0)
		sink (0)	sink (0)	sell all (0)
		storm drain (0)	storm drain (0)	trash (3)
		trash (0)	toilet (0)	other (0)
		no answer (2)	no answer (3)	no answer (0)
nuclear worms	3	give it to customers (2)	do not use SW (3)	give it to customers (0)
		ocean or bay (0)	ocean or bay (0)	return to dealer (1)
		sink (0)	sink (0)	sell all (0)
		storm drain (0)	storm drain (0)	trash (2)
		trash (0)	toilet (0)	other (0)
		no answer (1)	no answer (0)	no answer (0)
ghost shrimp	10	give it to customers (3)	do not use SW (3)	give it to customers (1)
		ocean or bay (0)	ocean or bay (2)	return to dealer (0)
		sink (2)	sink (2)	sell all (0)
		storm drain (1)	storm drain (0)	trash (0)
		trash (2)	toilet (1)	other** (4)
		no answer (2)	no answer (2)	no answer (0)

* Survey respondents answered that they dispose bloodworms (1) and pileworms (1) in a garden
** Survey respondents answered that they dispose ghost shrimp in a garden (3) or feed it to birds (1)
N equals the number of surveys that provided information about these processes
Individual bait shops may have indicated more than one method of disposal of packing material, seawater, or unsold bait (in some cases totals add up to more than N)

bait shop purchases and field collections north and south of Point Conception at 13 sites along the west coast of the United States. Ghost shrimp size distributions can be found in Figure 7. The overall sample was female-biased with 4,410 females and 2,926 males. The overall distribution of parasites infecting ghost shrimp purchased from bait shops and ghost shrimp collected in field populations is shown in Figure 8.

Parasites infecting imported ghost shrimp

To determine what parasites infect imported ghost shrimp I purchased 200 ghost shrimp quarterly (1,400 total) between June 2008 and January 2010 from two bait shops located in Los Angeles County. According to bait shop employees, all imported ghost shrimp were imported from Washington State with one exception: in summer 2009 sampling period ghost shrimp were imported from Oregon. The sample was female-biased with 878 females and 522 males. Approximately 18% of the females (158 out of 878) were brooding.

Of the 1,400 ghost shrimp inspected, 197 (14.07%) were infected with the bopyrid isopod *Ione cornuta* in one of their gill chambers. Typically, a female-male pair was found in one of the gill chambers, but in a few instances only the female was present. Isopods were usually alive, and most females carried either eggs or hatched epicaridium larvae. Overall, 12.8% of the females (112 out of 878) and 16.3% of the males (85 out of 522) were infected with *I. cornuta*. None of the 112 female ghost shrimp infected with *I. cornuta* were brooding. Of the 766 females not infected with *I. cornuta*, 158 (~20%) were brooding.

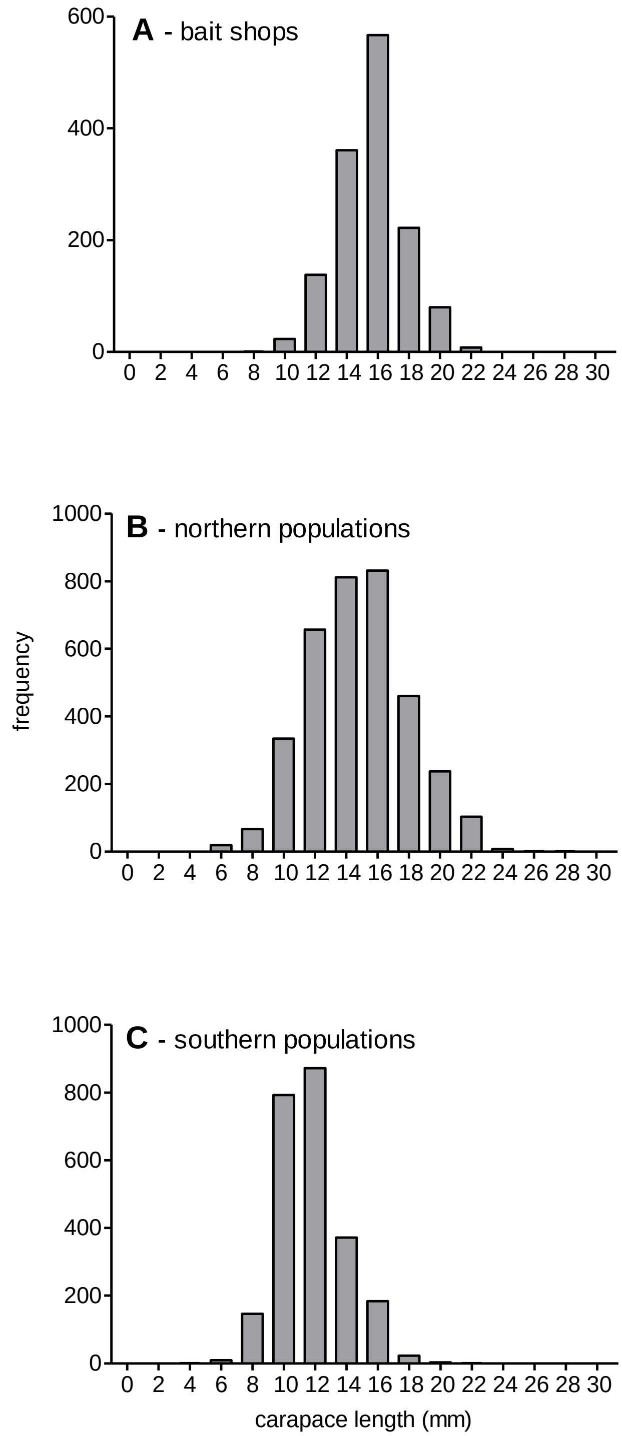

FIGURE 7. Size distribution of ghost shrimp purchased from bait shops (a), and collected in populations north (b) and south (c) of Point Conception.

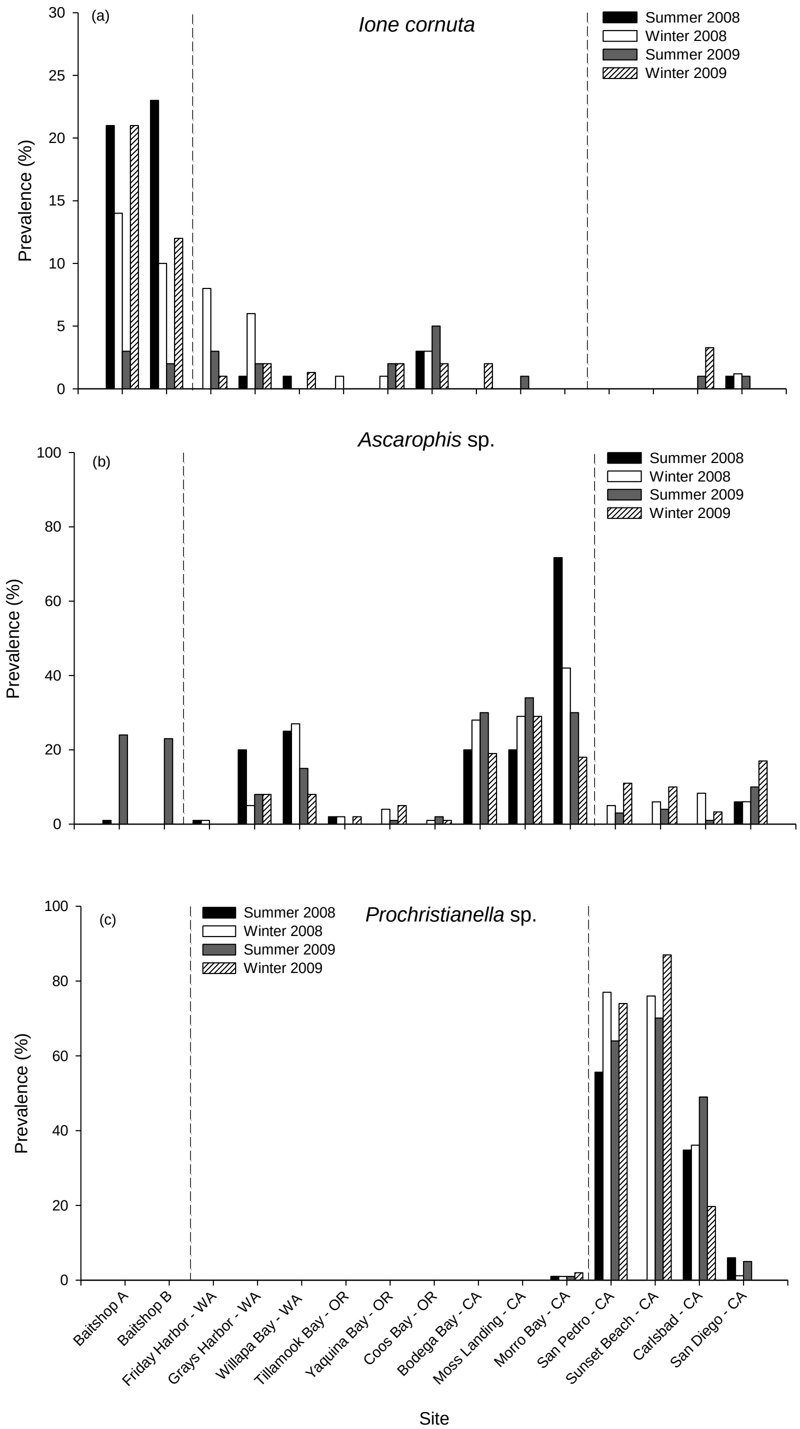

FIGURE 8. Overall distribution of *Ione cornuta* (a), *Ascarophis* sp. (b), and *Prochristianella* sp. (c) infecting ghost shrimp on the west coast of the United States. Vertical dashed lines separate bait shops, sites north of Pt. Conception, and sites south of Pt. Conception from left to right.

A total of 49 out of 1,400 (3.5%) imported ghost shrimp were infected with nematodes. These nematodes likely belong to the genus *Ascarophis* (A. Kuris, University of California, Santa Barbara, pers. comm.), although more than one species of nematode may infect ghost shrimp. Nematode prevalence was 5 times higher in females than in males. Overall, 5% of the females (44 out of 878) and 0.95% of the males (5 out of 522) were infected with nematodes. Almost half (45.5%) of the female ghost shrimp infected with nematodes were brooding. Of the 834 females not infected with *Ascarophis* sp., 138 (~16%) were brooding. Almost all (47 out of 49) ghost shrimp infected with nematodes were found in the summer 2009 sampling, the only occasion when ghost shrimp were reportedly imported from Oregon State.

No trypanorynch cestodes were found infecting imported ghost shrimp purchased at bait shops during the sampling period.

The symbiotic copepod *Clausidium vancouverense* was observed in many imported ghost shrimp purchased at local bait shops. These copepods are native to southern California and were also observed in natural populations of ghost shrimp in sites located both north and south of Point Conception. Furthermore, the effects, if any, of these copepods on the fitness of their hosts is unclear. In contrast, the importation of the parasitic bopyrid isopod *Ione cornuta* could be a reason of concern.

Ghost shrimp parasites distribution in the west coast of the United States

To determine the distribution of parasites that infect ghost shrimp in the west coast of the United States I collected ghost shrimp in thirteen field sites, nine of these sites are located north of Point Conception and four sites were located south of Point Conception.

Northern populations. I collected a total of 3,531 ghost shrimp from nine populations north of Pt. Conception quarterly between June 2008 and December 2009. The pooled sample was female-biased with 2,083 females and 1,448 males. Approximately 34% of the female ghost shrimp (715 out of 2,083) were brooding. Ghost shrimp populations were infected with *Ione cornuta* at 8 out of the 9 sites north of Pt. Conception. The exception was Morro Bay, the closest site to Pt. Conception, where no ghost shrimp were infected with *I. cornuta*. Of the 3,531 ghost shrimp collected at sites north of Pt. Conception, 47 (1.3%) were infected with *I. cornuta* in one of their gill chambers. This was significantly lower than the prevalence in imported ghost shrimp (Fisher's exact test, $P<0.001$). The smallest ghost shrimp infected with *I. cornuta* was a 7.6 mm carapace length female collected in Coos Bay, Oregon.

Nematodes were found infecting ghost shrimp at all sites north of Pt. Conception. The prevalence of nematodes in populations north of Pt. Conception was relatively high (14.2%). Of the 501 ghost shrimp infected with nematodes 316 were females and 185 were males. No difference in prevalence was observed between females and males (15.1% and 12.8%, respectively). Of the 316 infected female ghost shrimp, 132 (41.7%) were brooding while 583 out of 1767 (33%) of uninfected females were carrying broods. The smallest infected ghost shrimp was a male with a 7.6 mm carapace collected in Elkhorn Slough, central California.

The prevalence of trypanorynch cestodes infecting ghost shrimp populations north of Pt. Conception was very low (<0.15%). This trypanorynch species was identified as *Prochristianella* sp. using morphological characters (Dr. Robin Overstreet, University of Southern Mississippi, pers. comm.). All northern ghost shrimp infected with trypanorynchs were collected at Morro Bay, in central California. Of the five infected ghost shrimp, four were males and only one was a female; the female was the smallest of the infected ghost shrimp with a carapace length of 11.1 mm.

<u>Southern populations</u>. South of Pt. Conception I collected a total of 2,405 ghost shrimp from four sites. Collections were performed quarterly between June 2008 and December 2009. The sample was female-biased with 1,449 females and 956 males. In southern sites, 22.5% (259 out of the 1,449) of the female ghost shrimp were brooding. Of the 2,405 ghost shrimp collected at sites south of Pt. Conception, 11 (0.46%) were infected with *I. cornuta* in one of their gill chambers. Seven of the infected ghost shrimp were collected in San Diego and four in Carlsbad. Of the infected ghost shrimp seven were female and four were male. None of the infected female ghost shrimp were brooding. The smallest infected ghost shrimp was a 9.9 mm carapace length female collected in San Diego.

Nematodes were found infecting ghost shrimp at all sites in southern California with an average prevalence of 7.2%. Of the 173 infected ghost shrimp, 111 were females and 62 were males. I found no difference in the prevalence of nematodes between females and males (7.7% and 6.5%, respectively). Of the 111 infected female ghost shrimp, 16 (14.4%) were brooding while 243 out of 1,338 (18.2%) of uninfected females were carrying broods. The smallest ghost shrimp infected with nematodes in southern California was a male with a 9.0 mm carapace.

Nearly all (>99%) trypanorynchs collected in this study were found in ghost shrimp populations south of Pt. Conception. All sites in southern California had ghost shrimp infected with trypanorynchs with an average prevalence of 43% (1036 out of 2405). Of the 1036 ghost shrimp infected with *Prochristianella*, 664 were females and 372 were males. Of the infected females 15.5% were brooding. Twenty percent of uninfected females were brooding. The smallest infected ghost shrimp was a 7.2 mm carapace length male.

The prevalences of *Ione cornuta, Ascarophis* sp*.,* and *Prochristianella* sp. were different among sites located north and south of Point Conception, and among bait shops and sites located south of Point Conception (Table 5). I also observed differences in the prevalence of *Ascarophis* sp. and *Prochristianella* sp. between males and females. The prevalence of *I. cornuta* prevalence did not differ according to sex (Table 5). The percentage of brooding females between infected and uninfected ghost shrimp was different for all three parasites (Table 6). None of the females infected with *I. cornuta* were brooding, while 1,132 out of 4,261 uninfected females (26.6%) were brooding. In contrast, a higher percentage of brooding females was observed in ghost shrimp infected with *Ascarophis* sp. (35.7%) and *Prochristianella* (27.5%) in comparison to uninfected brooding females (24.5% and 15.5%, respectively).

A total of 2,486 ghost shrimp were identified as either *Neotrypaea californiensis* or *N. gigas*. Prior to fall 2009, only adult males were identified to species, by inspecting

TABLE 5. Comparison of the Prevalences of *Ione cornuta*, *Ascarophis* sp. and *Prochristianella* sp. Infecting Ghost Shrimp Between Sites Located North versus South of Point Conception, Bait Shops versus Sites Located North of Point Conception, Bait Shops versus Sites Located South of Point Conception, and Female versus Male Hosts

Comparison	Direction of Difference	Fisher's P-value
N vs. S of Pt. Conception		
Ione cornuta	N (1.3%) > S (0.43%)	0.001
Ascarophis sp.	N (14.2%) > S (7.2%)	< 0.001
Prochristianella sp.	N (0.15%) < S (43%)	< 0.001
bait shops vs. N of Pt. Conc.		
Ione cornuta	b.s. (14.1%) > N (1.3%)	< 0.001
Ascarophis sp.	b.s. (3.5%) < N (14.2%)	< 0.001
Prochristianella sp.	no sig. difference	0.330
bait shops vs. S of Pt. Conc.		
Ione cornuta	b.s. (14.1%) > S (0.43%)	< 0.001
Ascarophis sp.	b.s. (3.5%) < S (7.2%)	< 0.001
Prochristianella sp.	b.s. (0.0%) < S (43%)	< 0.001
females vs. males		
Ione cornuta	no sig. difference	0.603
Ascarophis sp.	F (10.7%) > M (5.1%)	0.004
Prochristianella sp.	F (15.1%) > M (12.9%)	0.008

TABLE 6. Comparson of the Percentage of Brooding Female Ghost Shrimp Infected versus Uninfected with *Ione cornuta*, *Ascarophis* sp. and *Prochristianella* sp.

Comparison	Direction of Difference	Fisher's P-value
infected vs. uninfected		
Ione cornuta	inf. (0.0%) < uninf. (26.6%)	< 0.001
Ascarophis sp.	inf. (35.7%) > uninf. (24.5%)	< 0.001
Prochristianella sp.	inf. (27.5%) > uninf. (15.5%)	< 0.001

their major claw. After fall 2009, every ghost shrimp collected, including females and juveniles, were identified by inspecting the shape of their eye stalks. In sites north of Point Conception, ghost shrimp were predominantly (98%) identified as *N. californiensis* while the distribution of the two species was much more even (52.3% *N. californiensis* and 47% *N. gigas*) in sites south of Point Conception. (Table 7). Parasite prevalence was different between the two species of ghost shrimp. *Ione cornuta* was only found infecting *N. californiensis*, with higher prevalence in sites north of Point Conception in relation to southern sites. Nematodes were found infecting both species of ghost shrimp, but *N. gigas* were only infected in southern sites. However, very few *N. gigas* were collected north of Point Conception. *Prochristianella* sp. also infected both species of ghost shrimp. This parasite was found almost exclusively in southern sites, with the exception of two infected *N. californiensis* in Morro Bay, California, approximately 60 miles north of Point Conception. In southern sites, *N. gigas* were infected with *Prochristianella* sp. at a much higher prevalence in comparison to *N. californiensis* (Table 8).

<u>Establishment: Survival of NIS Imported via the Live Bait Trade in Relation to Southern California Temperatures</u>

All species showed a high percent survival after five days in the two cooler temperature treatments (Figure 9). The percent survival of bloodworms was 95% at 12^{o}C and 90% at 16^{o}C. I observed a similar pattern for lugworms, with 97.5% and 90% of the animals surviving at 12^{o}C and 16^{o}C, respectively. Even though *Ione cornuta* were removed from their hosts and maintained in isolation in the experiment, we observed high

TABLE 7. Number of Individuals of Ghost Shrimp Identified by Morphological Characteristics either as *Neotrypaea californiensis* or *N. gigas* in Field and Bait Shop Collections

Species	Morphological character Claw *	Eyestalk **
Neotrypaea californiensis		
N. of Pt. Conception	676	1245
S. of Pt. Conception	166	372
Total	842	1617
Neotrypaea gigas		
N. of Pt. Conception	14	31
S. of Pt. Conception	149	309
Total	163	340

* Adult males only; identified throughout the study.

** Females, males, and juveniles; identified from September 2009 on.

TABLE 8. Prevalence (%) of *Ione cornuta*, *Ascarophis* sp. and *Prochristianella* sp. Infecting *Neotrypaea californiensis* and *Neotrypaea gigas* North and South of Point Conception (Number of infected individuals in parenthesis)

Species	*N*	*Ione cornuta*	*Ascarophis* sp.	*Prochristianella* sp.
Neotrypaea californiensis				
N. of Pt. Conception	1566	5.7 (90)	8.3 (130)	0.13*(2)
S. of Pt. Conception	454	1.1 (5)	13.7 (62)	18.3 (83)
Neotrypaea gigas				
N. of Pt. Conception	34	0 (0)	0 (0)	0 (0)
S. of Pt. Conception	414	0 (0)	8.2 (34)	76.8 (318)

* Collected at Morro Bay, central California.

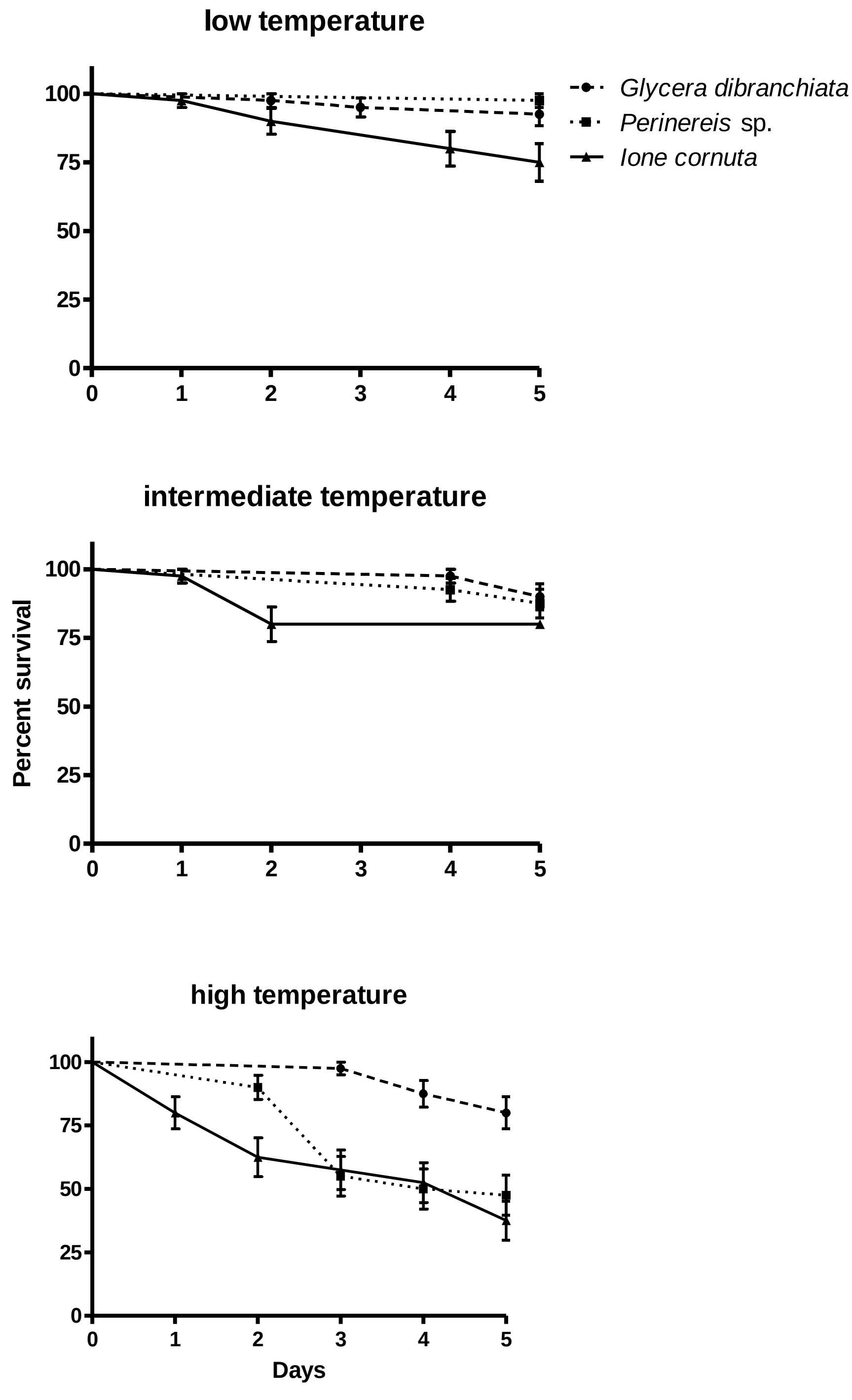

FIGURE 9. Survival curve of *G. dibranchiata*, *Perinereis* sp., and *I. cornuta* exposed to lower (top), intermediate (middle), and higher (bottom) temperatures typical of southern California over the course of the experiment.

survival for this species in the two cooler treatments. Seventy five percent of the *I. cornuta* survived at 12^{o}C, and 80% survived at 16^{o}C.

There was no significant difference in bloodworm survival among the three temperature treatments (F = 2.00, p = 0.178). For both *Perinereis* sp. and *Ione cornuta* there was no significant difference in survival between the two cooler treatments, but fewer individuals survived in the highest temperature treatment for the two species (ANOVA: F = 11.08, p = 0.005 and F = 9.733, p = 0.003, respectively) (Figure 10).

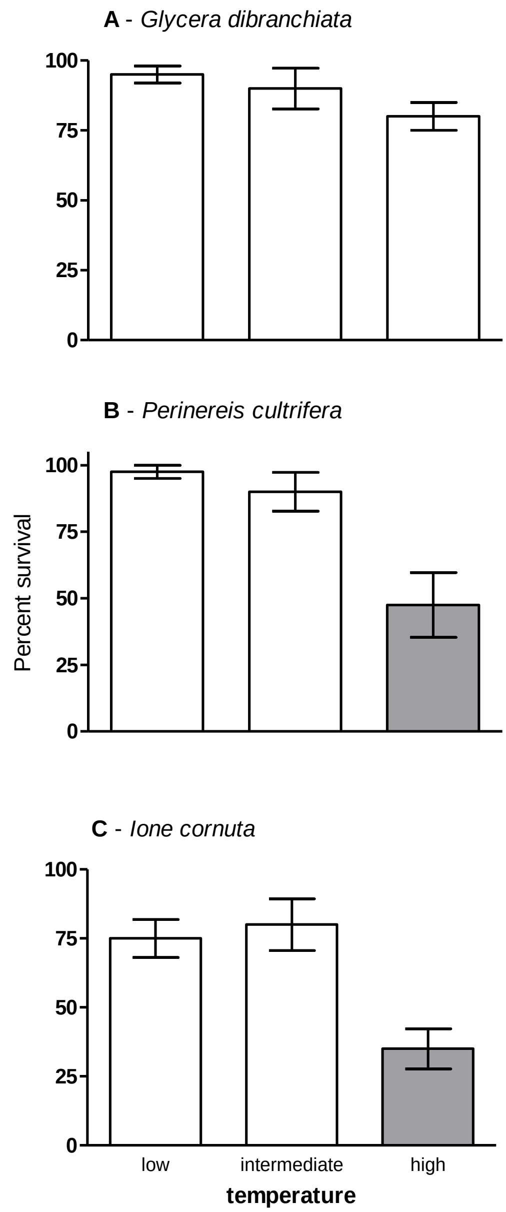

FIGURE 10. Average percent survival (±SE) of *G. dibranchiata*, *Perinereis* sp., and *I. cornuta* after exposed to southern California thermal conditions after five days. Gray bars indicate significant difference. No difference in survival was observed among the three temperature treatments for *G. dibranchiata* (F = 2.0, p = 0.178). There was a significant difference in survival of *Perinereis* sp. and *I.cornuta* among the treatments (F = 11.08, p = 0.005, and F = 9.733, p = 0.003, respectively).

CHAPTER 4

DISCUSSION

Transport and Release of Bait Species: Investigating the Numbers and Release Pathways of NIS Imported into California via the Marine Live Bait Trade

This study provides for the first time a state-wide estimate of the number of individuals of marine NIS imported into California annually via the live bait trade. This information helps determine the number of individuals of different species of live bait collected and transported by distributors from their native regions to live bait shops (Figure 1). I estimated that nearly 575,000 bloodworms, 600,000 pileworms, and almost two million ghost shrimp are imported into California annually. A previous study on hitchhiker species packed with baitworms estimated that over 600,000 bloodworms and 700,000 pileworms are imported each year into the San Francisco Bay Area from the U.S. East Coast (Cohen et al., 2001). It is difficult to compare these numbers because the studies were done almost ten years apart. It is possible, however, that in the last decade the number of bloodworms and pileworms imported into California has declined. Previous research on the Maine baitworm fisheries has shown that the number of landings of these species peaked between the 1960s and 1970s, then suffered a decline and was not expected to increase again in the near future (Brown, 1993).

Bait shops selling marine live bait seem to have high turnover. I compared the bait shop list in this study with the list in Cohen et al. (2001) and found that only a few of the shops listed in that previous study were still in business. Another difficulty in generating a complete list is the fact that online sources are updated frequently and new bait shops are added while others are removed from the database. I observed such changes in online sources within a six-month period.

There at least three sources of error associated with the estimates provided in this study. First, there may be businesses that are hard to find (e.g. liquor stores selling marine live bait) using the methods used in this study since they are not likely to be listed under “Fishing bait and tackle”. Second, not every bait shop identified as selling marine live bait participated in the survey, yielding a low sample size. Reasons could be that some bait owners perceived the survey as a potential threat to their business (e.g. could result in an increase in regulations), language difficulties since many bait shops are operated by people whose first language is not English, or due to a general lack of willingness to participate in surveys. Despite these shortcomings, our response rate is very similar to the response rates obtained in other studies using similar survey methods (Weigle et al., 2005). Third, not every survey respondent provided estimates of the number of individuals stocked annually.

These factors contribute to the error associated with the estimates provided in this study. In the future, one way to verify my estimates is to obtain numbers of individuals imported annually from marine live bait wholesalers and compare these data to the numbers estimated in this study. Previous research on freshwater live baitfish industry in the North-Central Region of the U.S. (Meronek et al., 1997) showed that numbers obtained from a survey with bait shops were 42% lower than those obtained from wholesalers. The numbers estimated in the present study suggest that the number of organisms imported into California annually is relatively high, and the live bait trade merits further investigation.

Besides numbers of individuals imported, this study provided other information that will help to determine the likelihood of introduction of NIS live bait species, hitchhikers, and parasites. Hitchhiker species were reported to be imported with every target species of live bait worm. At this point, the number of individuals of hitchhiker species has not been quantified. Seaweeds used as packing materials and hitchhiker species can be introduced with at least four species of Atlantic organisms reported to have temporarily or permanently established populations in the San Francisco Bay Area (Cohen et al., 2001). One of these species is the periwinkle snail *Littorina saxatilis*, which was likely introduced by anglers disposing seaweeds used as packing materials for baitworms in San Francisco Bay (Carlton and Cohen, 1998). Besides hitchhikers, parasites were also found to infect imported ghost shrimp (*Neotrypaea californiensis*) sold in bait shops in southern California.

This study estimated the numbers of individuals imported from donor regions to bait shops. To be introduced, however, species need to go through one more stage; the disposal of organisms by humans into the environment (Figure 1). Survey responses show that disposal of packing materials and unsold live bait happens through methods that may result in introduction of NIS into local marine habitats (e.g. directly disposed into ocean or given to customers that may dispose materials in ocean). Results suggest that anglers end up with a significant percentage of live bait species, seaweeds used as packing materials, and hitchhiker species. Currently, there are no studies that show that marine live bait is locally disposed in the environment by the anglers. However, this practice is common in freshwater systems (Litvak and Mandrake, 1993; Keller et al., 2007), and it is extremely likely that this type of practice also happens in marine environments. Future surveys conducted with anglers would be useful for determining the propagule pressure of NIS of marine live bait in California coastal habitats.

Survey results show that several marine species of live bait are imported into California year-round. According to survey results, most of these species have been imported for at least 30 years. Species of bait worms such as bloodworms, pileworms, and lugworms have a peak in sales between May and September. This trend is likely driven by an increase in recreational fishing activities in marine coastal areas during summer months. Overall, live ghost shrimp sales peaked in the winter, between November and March. This trend seems to be strongly influenced by the sturgeon fishing season in the Sacramento Delta Region where ghost shrimp are commonly used as bait for sturgeon fishing. However, if only bait shops in southern California are considered, ghost shrimp were reported to be sold year-round, with no particular time of year indicating a peak in sales.

Transport of Parasites: Estimating the Quantities of Parasites Imported into California via Ghost Shrimp Trade

This study also investigated parasites associated with ghost shrimp imported into California. The initial motivation for this investigation was the high prevalence of the bopyrid isopod *Ione cornuta* previously reported infecting imported ghost shrimp obtained from southern California bait shops (Pernet et al., 2008). Bopyrid isopods have the ability to castrate both male and female hosts (Munoz and George-Nascimento, 1999;

Astete-Espinozal and Caceres, 2000; McDermott, 2002). Consistent with this, none of the female ghost shrimp infected with this parasite in this study were brooding during the reproductive season. This shows that this parasite can negatively affect host fitness. This reduction in host-fitness is likely to have population-level effects. For example, Chapman et al. (2006) observed a drastic decline in populations of the mud shrimp *Upogebia pugettensis* following the introduction of the non-indigenous bopyrid isopod *Orthione griffenis* in northern estuaries. However, at this point, it is still unclear how severely *I. cornuta* affects ghost shrimp populations.

Although several lines of evidence suggest that the natural southern range limit of *I. cornuta* lies north of Point Conception (Pernet et al., 2008), in this study *I. cornuta* was observed infecting ghost shrimp in populations south of Point Conception, in Carlsbad and San Diego. This parasite was also found in the eight northernmost sites sampled, from Elkhorn Slough, in central California, to False Bay, in Washington. There was, therefore, a gap in distribution of this parasite of approximately 480 km extending from Elkhorn Slough to Carlsbad. Genetic analysis comparing eight specimens of *I. cornuta* found in northern populations and four specimens collected in southern populations showed that there is very little variation between northern and southern *I. cornuta* in 238 base pairs of the cytochrome B gene (A. Deconinck, M. Velarde, and B. Pernet, unpublished data). This lack of genetic differentiation could be explained by either of two hypotheses. First, the range of this species could be wider than initially described and populations in the west coast do not differ genetically across the distribution range analyzed in this study. Second, *I. cornuta* did not show genetic differences between northern and southern populations because this species may have been introduced to southern California from northern areas via the live bait trade. At this point, it is still unclear whether *I. cornuta* found in southern populations are native or non-indigenous.

The overall prevalence of *I. cornuta* infecting imported ghost shrimp was much higher than previously observed. Pernet et al. (2008) found bopyrids to infect imported ghost shrimp at an overall prevalence of 5.8%. The overall prevalence of *I. cornuta* infecting ghost shrimp purchased at bait shops in the present study was much higher, at 14.1%. This prevalence was also much higher than the overall prevalence observed in field populations north of Point Conception (1.3%). The reason for this difference is unclear at this point. One possibility is that I sampled larger ghost shrimp from bait shops than ghost shrimp from northern field populations. Previous research has shown that larger ghost shrimp are more likely to be infected by bopyrids (O'Brien and Van Wyk, 1985). Consistent with this, a logistic regression analysis with all ghost shrimp collected in this study shows that shrimp carapace length is positively associated with *I. cornuta* prevalence ($p < 0.001$). However, in this study, the size distribution of ghost shrimp purchased from bait shops is nearly identical to the size distribution of ghost shrimp collected in northern populations (Figure 7). Therefore, size alone cannot explain this difference in prevalence. In my field sampling I used an Alvey pump to collect ghost shrimp while bait shops reported that they use a large hose to blast ghost shrimp burrows with water. This difference in collection methods may contribute to the difference seen in prevalence.

Approximately 1,900,000 ghost shrimp were estimated to be imported annually into California. In this study, imported ghost shrimp were infected with *I. cornuta* with a prevalence of 14%. This suggests that approximately 266,000 bopyrid isopods are potentially imported annually with their ghost shrimp hosts. The majority of *I. cornuta* observed infecting ghost shrimp were brooding, and estimations suggest that each brooding female carries between 30,000 and 50,000 eggs or hatched larvae (B. Passarelli, unpublished data). This then indicates that 8-13 billion eggs (or hatched larvae) are imported along with ghost shrimp each year. Even if only a small percentage of these eggs are introduced to the environment, it would substantially increase the propagule pressure of *I. cornuta*. However, to better assess the risks that this parasite may pose to local populations of ghost shrimp, other aspects of the life cycle of *I. cornuta* need to be investigated, such as the presence of suitable intermediate hosts.

In the typical life cycle of bopyrid isopods, brooding females release epicaridium larvae. These attach to an intermediate host, usually a calanoid copepod, where they sequentially metamorphose into two other larval stages: the microniscus and the cryptoniscus. The cryptoniscus larvae leave the copepod and infect the final host (Anderson and Dale, 1981). If intermediate hosts are present in southern California, it is possible that the prevalence of *I. cornuta* could increase in ghost shrimp populations in southern California. Monitoring prevalence in local populations is crucial to detect increases in the number of ghost shrimp infected, which could in turn lead to a decline in ghost shrimp numbers, especially in areas where *I. cornuta* have recently been found.

Two other types of parasites, nematodes and cestode tapeworms, were also observed infecting ghost shrimp in southern California populations. The distribution and taxonomy of these parasites is not well known so there is a possibility that non-indigenous species, other than *I. cornuta*, are being brought to California with the ghost shrimp trade. Nematodes were not found infecting imported ghost shrimp, with one exception, in summer 2009. This was the sole occasion when bait shop workers reported that ghost shrimp were collected in Oregon, as opposed to Washington. This change in the collection site may explain the difference in nematode prevalence in imported ghost shrimp. In field collections, however, nematodes were found infecting ghost shrimp in at least one sampling event in all collection sites during the course of this study. This suggests that parasitic nematodes are widely distributed along the west coast of the United States. These nematodes most likely belong to the genus *Ascarophis* (A. Kuris, University of California Santa Barbara, pers. comm.). The taxonomy is poorly known, and maybe more than one species may be found infecting ghost shrimp. Depending on the native range of these species, it is possible that NIS of *Ascarophis* are imported with ghost shrimp via the live bait trade. At this point, we are uncertain of how many species of nematodes infect ghost shrimp and their respective distributions. DNA analysis is required to identify the number of species that are present. Nematodes do not seem to affect the fitness of female ghost shrimp (Table 6). However, *Ascarophis* sp. only use ghost shrimp as intermediate hosts, and use species of actinopterygian fishes as their final hosts. The effects these parasites have on their final hosts, and what species of fish serve as final hosts, are still unclear at this point. Although many questions regarding the nematode species and their final hosts remain unanswered, the introduction of NIS of *Ascarophis* sp. via the live ghost shrimp trade in California cannot be ruled out.

None of the imported ghost shrimp inspected in this study were infected with larval cestode tapeworms (*Prochristianella* sp.). The distribution of *Prochristianella* observed in this study was limited to southern California sites, with the exception of five specimens found in Morro Bay, the probable northern limit of their distribution. In sites south of Point Conception, *Prochristianella* was common, with an overall prevalence of 43.1%. Like *Ascarophis* sp., *Prochristianella* sp. use ghost shrimp as an intermediate host. The final hosts of other species belonging to the genus *Prochristianella* are various species of elasmobranchs. The ecological and physiological effects of these parasites, both on ghost shrimp and their final hosts are not well understood at this point. However, since the ghost shrimp trade imports individuals from Oregon and Washington, this particular parasite species is not being transported with the live ghost shrimp trade into California and is, therefore, not a species of concern.

All imported ghost shrimp identified by the major claw of adult males or eye stalk characters were *N. californiensis*. In contrast, both *N. californiensis* and *N. gigas* were identified in field populations. Differences were observed both in the geographic distribution within the study region and the prevalence of parasites in these two species of ghost shrimp. While almost all ghost shrimp north of Point Conception were identified as *N. californiensis*, in some sites south of Point Conception both species were observed. In northern populations, *N. californiensis* was the most common ghost shrimp species at all sites. Of the 1,092 ghost shrimp identified by morphological characters in northern sites, only thirty four were *N. gigas*. In southern populations, however, an almost equal number of ghost shrimp was identified as either *N. californiensis* (454) or *N. gigas* (414) out of the 868 individuals inspected for morphological differences. Both species of ghost shrimp were observed at all four southern sites. In Mission Bay and Agua Hedionda Lagoon most of the ghost shrimp observed were *N. californiensis*, while in Anaheim Bay almost all ghost shrimp observed were *N. gigas*. The population in Los Angeles Harbor, in San Pedro, was the only one where both species of ghost shrimp were observed in approximately equal numbers.

Only *N. californiensis* specimens were infected with *I. cornuta*. Therefore, sites in southern California such as Mission Bay, Agua Hedionda Lagoon, and Los Angeles Harbor have the potential to harbor ghost shrimp infected with *I. cornuta*. Thus, it is important to monitor these areas to detect any changes in *I. cornuta* prevalence which could result in a decline in ghost shrimp populations. *Ascarophis* sp. were observed infecting both *N. californiensis* and *N. gigas* in field populations, with a higher prevalence in sites south of Point Conception. However, the prevalence of *Ascarophis* sp. in imported ghost shrimp was very small in relation to field populations of ghost shrimp. Therefore, it is unlikely that imported ghost shrimp released into local habitats would result in the introduction of this particular species.

Establishment: Survival of NIS Imported via the Live Bait Trade in Relation to Southern California Temperatures

Introduction is only the first step in the invasion process. Many factors, such as physiological limits and community interactions, may affect the likelihood of a species becoming established. I evaluated one of these: survival in relation to short term exposure to temperatures found in southern California. The approach used in this study was different from typical temperature tolerance experiments, as no time was given for acclimation. This was done to simulate the disposal of bait into marine habitats. My results show that all three NIS used in this study, *Glycera dibranchiata*, *Perinereis* sp. and *Ione cornuta*, are able to survive at least five days following exposure to temperatures typically found in southern California coastal habitats (12^{o}C-24^{o}C). Moreover, they show that exposing live bait to different temperatures without acclimation, which simulates what happens when leftover live bait is disposed in water, does not seem to significantly affect survival.

Glycera dibranchiata showed a relatively high and constant survival at all tested temperatures. *Perinereis* sp. and *I. cornuta* showed lower survival at the 24^{o}C treatment in comparison to the two colder treatments. However, even at the warmest temperature nearly 40% of the individuals survived after five days. Temperatures above 20^{o}C are only found in southern California in shallow habitats, such as estuaries and lagoons, and those types of habitat represent a small fraction of the total area where recreational fishing occurs. This suggests that establishment of the species tested in this study are likely to occur since thermal conditions found in most habitats in southern California do not seem to restrict their short term survival.

Ione cornuta showed high survival even after being removed from their hosts. Although this study showed that fully developed *I. cornuta* can survive in local temperature conditions in isolation of their hosts, it is very unlikely that an adult isopod can directly infect local ghost shrimp (Markham, 1992). Since it is still unknown if this species is native to southern California, further studies should investigate if the larval stages of *I. cornuta* can survive in southern California temperature conditions. In addition, it needs to be determined if the required intermediate hosts are present in order for larvae to fully develop into adults which would in turn be able to infect local ghost shrimp.

A study of the aquarium trade in the San Francisco Bay area using a similar approach to this study showed that five species of exotic fish are able to survive in temperatures typical of the Bay-Delta Region (Chang et al., 2009). This study indicated that species imported with the live bait trade can survive in local habitats and suggests that this pathway also merits further investigation. In this study, however, I assessed only one factor, short-term thermal tolerance, to determine the ability of non-indigenous bait and associated species to invade local habitats. Other organismal traits (e.g. long term survival, reproductive physiology) as well as ecological factors (e.g. availability of specific food, absence of controlling predators and parasites) are also important in determining risks of invasion (Coulatti et al., 2006) and should be examined. At this time, however, we know that species are imported year-round in large quantities and are able to survive in local conditions. However, predicting which species are going to become established is nearly impossible due to the number of factors involved in such predictions.

CHAPTER 5

CONCLUSIONS

This study provided previously unavailable data about the marine live bait trade in California and indicates that this trade cannot be ruled out as an introduction pathway of NIS. The results in this study show that hundreds of thousands of bloodworms, pileworms, and ghost shrimp are imported annually into California, and that importation of these species takes place year-round. Besides target species, hitchhikers and parasites are also imported and could potentially be introduced into local habitats. Furthermore, the species tested in this study were able to survive sudden temperature changes from the containers they are sold in to the experimental temperature treatments. These data suggest that NIS imported with the live bait trade could become established if introduced to local habitats. However, further research is necessary to calculate the propagule pressure of NIS (see Cohen et al., 2007) of marine live bait in local habitats by estimating the percentage of live bait that is discarded into the environment by anglers and bait shops. Also, long-term temperature tolerance should be performed with the species mentioned in this study and with larval stages of *I.cornuta*.

If the percent of live bait discarded by anglers in the marine environment is similar to the percent reported for the freshwater environment (Meronek et al., 1997), it is likely that marine species introduced to local habitats may become established. The estimated number of organisms imported annually into California, the year-round nature of the trade, the reported presence of hitchhiker species in the shipment of live bait, the importation of potentially non-indigenous parasites in the ghost shrimp trade, and the relatively high survival of individuals observed in the temperature tolerance experiment strongly suggest that species imported via the live bait trade can become established in southern California habitats.

The potential risks associated with the live bait trade could be reduced by adopting measures such as using labels that identify marine live bait species and place of origin, educating bait shop owners and customers about risks posed by the release of these NIS, and providing information about what safest methods of disposal of packing materials, seawater, and unwanted bait. In the case of ghost shrimp, *Ione cornuta* could be easily removed from their host and properly disposed of prior to using ghost shrimp as bait. This analysis will be critical in determining what management approaches should be taken to make the live bait trade in California as environmentally and economically safe and sustainable as possible.

APPENDICES

APPENDIX A

LIVE MARINE BAIT SURVEY

APPENDIX A

LIVE MARINE BAIT SURVEY (one sheet per species you sell)

1. This page is about (circle one): *Bloodworms* *Pileworms*

Lugworms *Nuclear worms* *Ghost shrimp* *Grass shrimp*

Other marine bait species (please specify) ____

2. How do you obtain this species?

Collect it yourself *In-state dealer* *Out-of-state dealer* *Other* _____

3. Where is the species collected (to the best of your knowledge)?

Country ___________________ State ___________________ City _____________

4. When did you start selling this species at your store?

Year ___________________

5. About how many individuals of this species do you stock (either collect yourself, or purchase from dealer) EACH YEAR? If you measure your stock in some other way (e.g., by the pound, or by the box), please write the number and units (pounds, boxes, etc.) and, if possible, provide a conversion to number of individuals.

Number of individuals stocked per year ___________________

6. Is there a time of the year when you sell this species the most (circle all that apply)?

Jan *Feb* *Mar* *Apr* *May* *Jun* *Jul* *Aug* *Sep* *Oct* *Nov* *Dec*

7. Since you've been selling this species, have you noticed any trends in sales (circle one)?

No trend *Increasing sales* *Decreasing sales*

8. When you receive this species into your store, is it in any kind of packing material, and if so, what material (circle all that apply)?

No packing material *Seawater* *Seaweeds* *Newspaper* *Other* _______________

9. If the species arrives in packing material, what do you do with the packing material (circle all that apply)?

No packing material *Give to customers with bait* *Into trash/landfill*

Down sink or toilet *Down storm drain (e.g. curbside)* *Into ocean or bay*

Bleach and discard in ocean or bay *Other* _____________________

10. Do you ever notice other animals or plants in the shipments with the packing materials or on the bait itself (circle all that apply)?

Never see other animals or plants *Snails* *Clams* *Worms* *Barnacles*

Crabs *Shrimp* *Seaweeds* *Other* _______________________

11. If you keep these animals in a seawater tank in your store, how do you discard used seawater (circle one)?

Don't use seawater tank *Down sink or toilet* *Down storm drain (e.g. curbside)*

Into ocean or bay *Bleach and discard in ocean or bay* *Other* _____________________

12. How do you usually dispose of unsold, damaged or dead individuals (circle one)?

Into trash/landfill *Down sink or toilet* *Down storm drain (e.g. curbside)*

Into ocean or bay *Other* __________________________

If you sell other species of live marine bait, please fill out a survey sheet for each of the species that you sell. If you do not sell any other type of live marine bait, you're done!

Thank you for your time & consideration.

APPENDIX B

LIVE MARINE BAIT SURVEY COVER LETTER

APPENDIX B

Dear bait shop owner or manager,

Recreational fishing is an important leisure and economic activity in California. One way to ensure that the industry remains strong is to minimize any negative impacts on the marine environment. I am a biology graduate student at California State University interested in learning more about this topic.

Non-native species are a growing threat to the marine habitats of California. You've probably heard about some non-native species that have been introduced into our state, like the green crab and the aquarium alga *Caulerpa*. Once introduced, non-native species are difficult and expensive to control. Most have been introduced by ballast water from commercial ships or oyster farming. I am interested in learning about other industries that work with live marine species.

I've prepared a short survey, which I'm sending to shops in California that sell live bait. The goal of this study is to better understand the trade and assess any risk of accidentally introducing non-native species into our coastal waters.

Please take a few minutes to answer this survey within the next 2 weeks, if possible. The survey consists of a sheet of questions (2 sides) to be filled out for **each live bait species** that you sell. I've enclosed 7 sheets. If you sell more than 7 live bait species, please contact me for additional survey sheets, or photocopy one of the sheets that you have. This survey is confidential; there is no need for you to write your business name or address on the form.

When you are finished, please return the completed survey in the enclosed, pre-paid envelope.

I thank you in advance for your help – it is critically important to ensuring that the live bait trade in California is as environmentally and economically safe and sustainable as possible.

Sincerely,

Bruno Passarelli, Graduate Student
Department of Biological Sciences
California State University, Long Beach
1250 Bellflower Blvd (Mailstop 3702)
Long Beach, CA 90840-3702

LITERATURE CITED

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Anderson G, Dale WE. 1981. *Probopyrus pandalicola*: morphology and development of larvae in culture. Crustaceana 41:143-161.

Astete-Espinozal LP, Caceres, CW. 2000. Effects of parasitism on nutritional physiology of the ghost shrimp *Neotrypaea uncinata* parasitized by the isopod *Ionella agassizi*. Rev Chil Hist Nat 73(2):243-252.

Bhaud M, Cha JH, Duchesne JC, Nozais C. 1995. Influence of temperature on the marine fauna: What can be expected from a climatic change. J Therm Biol 20 (1-2): 91-104.

Brown, B. 1993. Maine's baitworm fisheries: resources at risk? Am Zool 33:568-577.

Brusca RC, Coelho V, Taiti S (2001) A guide to the coastal Isopods of California. Internet address: http://tolweb.org/notes/?note_id=4180.

Carlton JT, Geller JB. 1993. Ecological Roulette: The global transport of non indigenous marine organisms. Science 261(5117):78-82.

Carlton JT, Cohen AN. 1998. Periwinkle's progress: The Atlantic snail *Littorina saxatilis* (Mollusca: Gastropoda) establishes a colony on Pacific shores. Veliger 41(4): 333-338.

Chang AL, Grossman JD, Spezio TS, Weiskel HW, Blum JC, Burt JW, Muir AA, Piovia-Scott J, Veblen KE, Grosholz ED. 2009. Tackling aquatic invasions: Risks and opportunities for the aquarium fish industry. Biol Invasions 11(4):773-785.

Chapman J, Dumbauld BR, McCoy L, Smith A, Markham, JC, Itani G. 2006. How mud shrimp *Upogebia pugettensis* got big new bumps. Pacific estuarine research Soc Ann Mtg abstract (www.pers-erf.org/PERS_2006_Abstracts.pdf) retrieved March: 13, 2008.

Cohen AN, Carlton JT. 1995. Nonindigenous Aquatic Species in a United States estuary: A case study of the biological invasions of the San Francisco Bay and Delta, University of California at Berkeley, Williams College-Mystic Seaport.

Cohen AN, Carlton JT, Fountain MC. 1995. Introduction, dispersal and potential impacts of the green crab *Carcinus maenas* in San Francisco Bay. Mar Biol 122:225-237.

Cohen AN, Carlton JT. 1997. Transoceanic transport mechanisms: Introduction of the Chinese mitten crab, *Eriocheir sinensis,* to California. Pac Sci 51 (1):1-11.

Cohen AN, Weinstein A, Emmett MA, Lau W, Carlton JT. 2001. Investigations into the introduction of non-indigenous marine organisms via-the cross continental trade in marine bait worms. Report for U.S. Fish and Wildlife Service, San Francisco Bay Program.

Cohen J, Mirotchnick N, Leung B. 2007. Thousands introduced annually: the aquarium pathway for non-indigenous plants to the St Lawrence Seaway. Front Ecol Environ 5(10):528.

Colautti RI, MacIsaac, HJ. 2004. A neutral terminology to define 'invasive' species. Divers Distrib 10:135-141.

Colautti RI, Grigorovich IA, MacIsaac HJ. 2006. Propagule pressure: a null model for biological invasions. Biol Invasions 8:1023-1037.

Dalton R. 2001. Action urged to combat killer algae. Nature 412:260.

Dawson MN. 2001. Phylogeography in coastal marine animals: a solution from California? J Biogeogr 28:723–736.

Dittel AI, Epifanio CE, 2009. Invasion biology of the Chinese mitten crab *Eriochier sinensis*: A brief review. J Exp Mar Biol Ecol 374 (2):79-92.

Duggan IC, Rixon CM, MacIssac HJ. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biol Invasions 8 (2):377-382

Frisch SM, Murray SN. 2002. The availability of species of *Caulerpa* and 'live rock' in retail aquarium outlets in southern California. In: Williams E, Grosholz E (eds) International *Caulerpa taxifolia* Conference Proceedings. California Sea Grant College Program, San Diego, California, p. 141-156.

Grosholz ED, Ruiz GM, Dean CA, Shirley KA, Maron JL, Connors PG. 2000. The impacts of a nonindigenous marine predator in a California bay. Ecology 81 (5): 1206-1224.

Keller RP, Lodge DM. 2007. Species invasions from commerce in live aquatic organisms: problems and possible solutions. Bioscience 57(5):428-436.

Kuris AM, Culver CS. 1999. An introduced sabellid polychaete pest infesting cultured abalones and its potential spread to other California gastropods. Invertebr Biol 118 (4):391-403.

Lau, W. 1995. Importation of baitworms and shipping seaweed: vectors for introduced species? In: Sloan, D., M. Christensen and D. Kelso (eds.), *Environmental Issues: From a Local to a Global Perspective*, Environmental Sciences Group Major, University of California, Berkeley, CA p. 21-38.

Leung B, Mandrak NE. 2007. The risk of establishment of aquatic invasive species: joining invasibility and propagule pressure. Proc R Soc B 274:2603-2609.

Levine JM, D'Antonio CM. 2003. Forecasting Biological Invasions with Increasing International Trade. Conserv Biol 17(1):322–326.

Litvak MK, Mandrake NE. 1993. Ecology of freshwater baitfish use in Canada and the United States. Fisheries 18(12):6-13.

Lockwood JL, Cassey P, Blackburn T. 2005. The role of propagule pressure in explaining species invasions. Trends Ecol Evol 20:223-228.

Ludwig HR, Leitch JA. 1996. Interbasin transfer of aquatic biota via angler's bait buckets. Fisheries 21(7):14-18.

Markham JC. 1992. The Isopoda Bopyridae of the Eastern Pacific - missing or just hiding? San Diego Soc Nat Hist 17:1–4.

McDermott JJ. 2002. Relationship between the parasitic isopods *Stegias clibanarii* and *Bopyrissa wolfii* and the intertidal hermit crab *Clibanarius tricolor* in Bermuda. Ophelia 56:33-42.

Meronek TG, Copes FA, Coble DW. 1997. A survey of the bait industry in the North-Central region of the United States. N Am J Fish Manage 17:703-711.

Munoz G, George-Nascimento M. 1999. Reciprocal reproductive effects in the symbiosis between ghost shrimp and bopyrid isopods at Lenga, Chile. Rev Chil Hist Nat 72:49-56.

O'Brien J, Van Wyk P. 1985. Effects of crustacean parasitic castrators on growth of crustacean hosts. In: Wenner A (ed) Crustacean issues 3. Factors in Adult Growth, A.A. Balkema, Rotterdam, pp 191–218.

Padilla DK, Williams SL. 2004. Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. Front Ecol Environ 2:131-138.

Pernet B, Deconinck A, Archie JW. 2008. Evaluating risks associated with transport of the ghost shrimp *Neotrypaea californiensis* as live bait. Mar Biol 163(6):1127-1140.

Pernet B, Deconinck A, Haney L. 2010. Molecular and morphological markers for distinguishing the sympatric intertidal ghost shrimps *Neotrypaea californiensis* and *Neotrypaea gigas* in the eastern Pacific. J Crustacean Biol 30 (2):159-166.

Pico AM, Collins JP. 2008. Amphibian commerce as a likely source of pathogen pollution. Conserv Biol 22 (6):1582-1589.

Portner HO. 2001. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Phys A 132: 739-761.

Rozsa L, Reiczigel, J, Majoros, G. 2000. Quantifying parasites in samples of hosts. J Parasitol 86:228-232.

Ruesink JL, Parker IM, Groom MJ, Kareiva PM. 1995. Reducing the risks of nonindigenous species introductions. Bioscience 45:465–477.

Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes and biases. Annu Rev Ecol Syst 31:481-531.

Semmens BX, Buhle ER, Salomon AK, Pattengill-Semmens CV. 2004. A hotspot of non-native marine fishes: evidence for the aquarium trade as an invasion pathway. Mar Ecol Prog Ser 266:239-244.

Simberloff D. 2009. The role of propagule pressure in biological invasions. Annu. Rev. Ecol. Evol. Syst. 40:81-102.

Thompson JA, Alam SK. 2005. Analysis of customs trade data to characterize importation of live bait. Fisheries 30(6):36-39.

Torchin ME, LaVerty KD, Kuris AM. 2002. Parasites and marine invasions. Parasitology 124:S137–S151.

Weigle SM, Smith LD, Carlton JT, Pederson, J. 2005. Assessing the risk of introducing exotic species via the live marine species trade. Conserv Biol 19:213-223.

Williamson M. 1996. Biological Invasions. Chapman Hall, London UK, 244 pp.

Wonham MJ, Carlton JT, Ruiz GM, Smith LD. 2000. Fish and ships: relating dispersal frequency to success in biological invasions. Mar Biol 136:1111-1121.

Zaleski SF., Murray SN. 2006. Taxonomic diversity and geographic distributions of aquarium-traded species of *Caulerpa* (Chlorophyta: Caulerpaceae) in southern California, USA. Mar Ecol Prog Ser 314:97-108.