

## Research Article

## The marine live bait trade as a pathway for the introduction of non-indigenous species into California: patterns of importation and thermal tolerances of imported specimens

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### Abstract

Importation of marine live bait may be an important pathway for the introduction of non-indigenous species (NIS), but little is known about the diversity of species or the numbers of individuals imported via this pathway. In 2009, we investigated the marine live bait trade in California as a potential introduction pathway for NIS. We estimated that in that year, ~ 1,900,000 ghost shrimp (*Neotrypaea californiensis*), ~ 575,000 bloodworms (*Glycera dibranchiata*), ~ 600,000 pileworms (*Alitta virens*), and ~ 1,100,000 lugworms (*Perinereis* sp.) were imported into California from different parts of the world. Along with the target imported species, hitchhiker species were commonly observed in live bait shipments. We exposed two of the live bait species (*G. dibranchiata* and *Perinereis* sp.) to a range of temperature conditions (12, 16, and 24 °C) found in nearshore marine habitats of southern California, and found that their short-term survival was not restricted at the two cooler temperature conditions, but that survival of *Perinereis* sp. was significantly reduced at the highest temperature, 24 °C. Though relatively few bait species were imported into the state in 2009, the large number of individuals imported and their high survival in thermal conditions typical of southern California habitats suggests that the live bait trade may be an important potential pathway for the introduction of NIS to this region.

**Key words:** baitworms, ghost shrimp, invasive, invasion vector, propagule pressure

### Introduction

Human-mediated introductions of marine non-indigenous species (NIS) to novel geographic areas occur through numerous vectors or mechanisms (Williams et al. 2013). In coastal marine systems, two pathways associated with commercial shipping—ballast water transport and hull fouling—are particularly important in terms of the diversity and numbers of individuals introduced to novel regions, and these pathways have been studied intensively over the past few decades (e.g., Ruiz et al. 2011; Davidson and Simkanin 2011; Seebens et al. 2013; Williams et al. 2013; Lacoursière-

Roussel et al. 2016). In ballast water transport and hull fouling, both transport and release of NIS are unintentional byproducts of shipping-related activities. However, there are a number of other introduction mechanisms where marine organisms are intentionally transported alive for commercial purposes. In comparison to ballast water and hull fouling vectors, these other introduction pathways, which include the seafood, aquaculture, aquarium, and live bait trades, have received relatively little attention by researchers (but see Cohen et al. 2001; Naylor et al. 2001; Chapman et al. 2003; Weigle et al. 2005; Chang et al. 2009; Kilian et al. 2012; Grosholz et al. 2015; Font et al. 2018).

These different trades associated with the transport of live organisms undoubtedly vary not only in the diversity and numbers of organisms transported, but also in the risk of their release and establishment into novel areas. These risks seem especially high for the live bait trade (Fowler et al. 2016). In this pathway, organisms are collected in one area, transported to another, then used alive in a habitat that is generally similar to that in which they were originally collected (Ludwig and Leitch 1996; Lau 1995; Cohen et al. 2001). One might imagine that only physiologically tolerant species that can survive the importation process are brought in via this mechanism, perhaps increasing the likelihood that members of these species can survive when released into the new habitat. Further, live bait are typically shipped in ways that maximize their survival (Blakeslee et al. 2016; Fowler et al. 2016). Studies on live bait transported in algal packing material indicate that a large number (> 50 taxa) of hitchhiker species may also be imported in the packing material (Cohen et al. 2001; Haska et al. 2011; Cohen 2012; Blakeslee et al. 2016; Fowler et al. 2016). A few studies suggest that anglers often release unused living bait and other organisms (e.g. algae and invertebrates packed with baitworms) directly into the novel area (Litvak and Mandrak 1993; Lau 1995; Kilian et al. 2012; Arias et al. 2013; Anderson et al. 2014). Together, these observations suggest that transport of live bait leads to a real possibility of the introduction of NIS, as has been confirmed by several studies in the past decade (Haska et al. 2011; Blakeslee et al. 2016; Fowler et al. 2016). In California, for example, at least four species of marine organisms are thought to have been introduced from the east coast of the United States in shipments of baitworms and their packing material (Cohen et al. 2001; Miller et al. 2004). This is likely an underestimate, as small, cryptic organisms that might have been introduced via this pathway may be difficult for researchers to detect (Cohen et al. 2001; Fowler et al. 2016).

To better understand and manage the risks of establishment of NIS into marine environments, it is important to quantify the magnitude of this potential pathway for their introduction. Minimally, this requires information about the diversity and numbers of bait species imported into

a region. Previous research (Cohen et al. 2001) and our preliminary observations have shown that several NIS are sold in bait shops in California as live bait for recreational marine fishing. Bloodworms *Glycera dibranchiata* Ehlers, 1868 (Glyceridae) and pileworms *Alitta virens* (M. Sars, 1835) (Nereididae) are imported into California from the northeast coast of the United States. Nereidids belonging to two species are imported from Asia: nuclear worms *Namalycastis* sp. Hartman, 1959, and lugworms *Perinereis* sp. Kinberg, 1865 (note that most marine biologists use the common name “lugworm” to refer to arenicolid polychaetes; in southern California bait shops, however, that common name refers to the nereidid *Perinereis* sp.) Another commonly sold species of marine live bait, the ghost shrimp *Neotrypaea californiensis* (Dana, 1854) (Callianassidae), is imported to California from Oregon and Washington. Though *N. californiensis* occurs naturally in California, imported individuals are sometimes infected with a parasitic isopod, *Ione cornuta* Bate, 1864 (Ionidae), that may be non-indigenous to southern California (Brusca et al. 2001; Pernet et al. 2008). Although permits are technically required to import live bait into the state (California Administrative Code, Title 14, §236(c)(6)(B)-(G)), in practice, no permits for importation of live bait appear to have been issued between 2004–2011 (Cohen 2012), a period of time in which live bait was certainly imported into the state (e.g., see our results below). Thus, reports on many aspects (e.g. number of imported species, seasonality, number of bait shops) of the live bait trade in California were not available from official sources at the time of this study. Information on these topics would be very useful in the managing the risks associated with this trade.

In this study, we characterize the marine live bait trade in California using an alternative source of data. In 2009, we conducted a state-wide survey of bait shops selling marine live bait, aiming to identify species imported into the state as live bait, the number of individuals imported annually, seasonal patterns of importation, and patterns of disposal of unsold live bait. In addition, we measured short-term survival of two of these species when exposed to a range of temperature conditions found in one of the habitats where these live bait were likely used by fishers: nearshore marine habitats of southern California.

## Materials and methods

### *Survey of the marine live bait trade in California*

We surveyed businesses selling marine live bait in California in 2009 about the identities, quantities, and fates of NIS imported for use as marine live bait. To identify the survey pool, we searched two online directories ([www.baitnet.com](http://www.baitnet.com) and [www.superpages.com/yellowpages](http://www.superpages.com/yellowpages)) for businesses listed as selling fishing bait and tackle in the 20 counties in California with coastal access or in the three counties in the Sacramento Delta region. We

included the latter because a marine species (the ghost shrimp *Neotrypaea californiensis*) is used in this area as bait in the recreational sturgeon fishery. We tested our assumption that businesses in inland counties did not sell marine live bait by calling ten randomly-selected bait-selling businesses in inland counties and asking if they sold marine live bait; all of these reported that they did not sell marine live bait. We then contacted each of the coastal/Sacramento Delta businesses by telephone and asked if they sold marine live bait, and conducted a mail survey of the businesses that did (Appendix 1). This consisted of 12 questions about the types of marine live bait sold, the amount sold, seasonal patterns in sales, packing materials, and the mode of disposal of unsold bait. We mailed surveys with a signed cover letter and a pre-stamped, addressed return envelope, and contacted recipients by telephone 10–14 days later to encourage return of questionnaires.

To estimate the number of individuals of each live bait species imported annually, we first estimated the number of businesses selling a particular bait species by multiplying the fraction of survey respondents selling that species by the total number of businesses identified as selling marine live bait. We then multiplied the result by the average number of individuals imported annually per responding business selling that species. Nonparametric bootstrap confidence intervals (percentile method, 1,000 bootstrap replicates) were calculated in R v.3.3.2 (R Core Team 2016) for the average number of individuals imported annually per bait shop.

#### *Survival of marine live bait in relation to temperature*

In an effort to understand the risks of establishment of some of these imported species should they be released into coastal marine environments of southern California, we assessed their short-term survival in relation to a range of temperatures found in nearshore marine habitats in that region. These experiments were carried out at Cabrillo Marine Aquarium in San Pedro, California, between July and September 2009. The experimental apparatus consisted of three water baths, each holding 16 1L plastic containers filled with aerated 33 psu filtered (20 microns sand filter and UV sterilizer) seawater obtained from a well on outer Cabrillo Beach. Using chillers or heaters, we maintained each of the three water baths at a different temperature close to low (~ 12 °C), intermediate (~ 16 °C) or high (~ 24 °C) temperatures typically found in southern California marine coastal habitats. We chose this temperature range to test if live bait purchased in southern California could survive if potentially introduced to local marine habitats. We insulated all water baths with foam (2.5–3.8 cm in thickness) to minimize temperature fluctuations caused by variation in external temperature. Temperature was measured at 30-min 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.

In this experiment, we tested the short-term survival of two species of imported baitworms (bloodworms, *Glycera dibranchiata*, and lugworms, *Perinereis* sp.). The other two species of imported baitworms (pileworms *Alitta virens* and nuclear worms *Namalycastis* sp.) usually sold at local bait shops were not available for sale at the time of the experiment. Baitworms were purchased at two bait shops near Long Beach, California on the day the experiment started. At these shops, both species were held in refrigerated cases at ~ 14 °C. Immediately after purchase, bait was rapidly (~ 1 hr) transported to Cabrillo Marine Aquarium in coolers so as to maintain temperature near bait shop holding temperatures. We randomly distributed living individuals of *G. dibranchiata* and *Perinereis* sp. individually into eight containers in each of the three water baths. Animals were exposed to experimental conditions within two hours after purchase. Specimens were not acclimated before they were exposed to experimental conditions, 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 the treatment temperature.

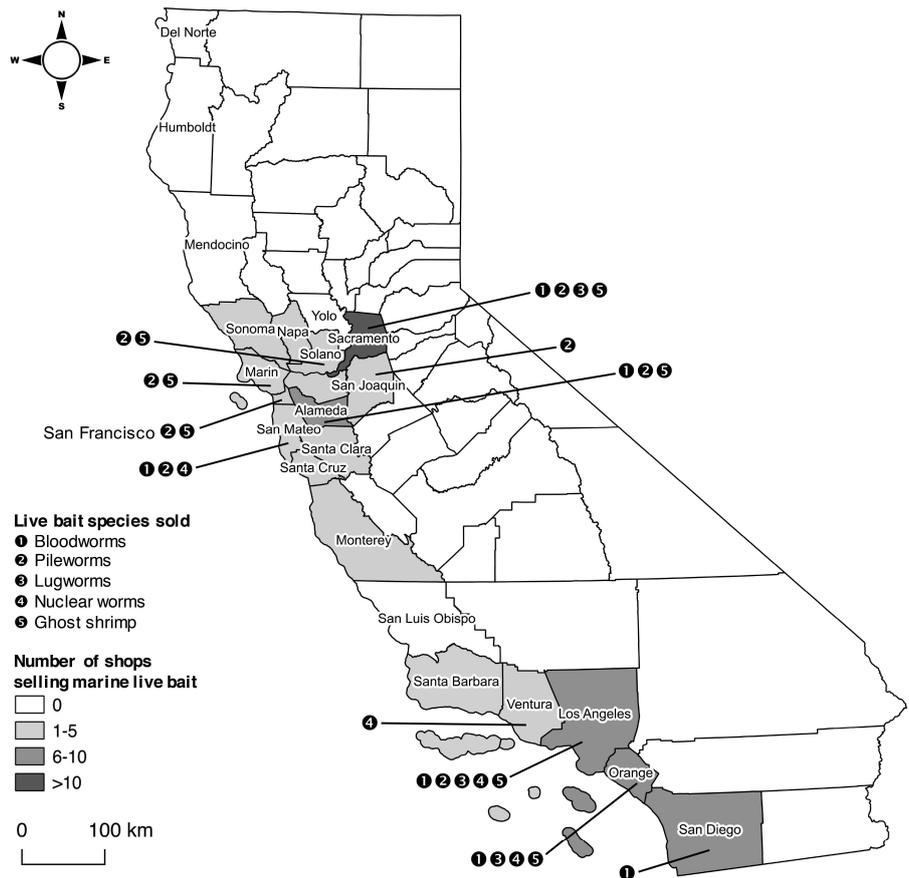
We assessed survival, as indicated by movement, at 24-hr intervals throughout the experiment. The experiment was replicated five times (trials 1–5), with a total of forty observations for each bait species at the three temperature treatments. Data were pooled for each species across the five trials. Data for all three species were normally distributed with homogeneous variances. Survival at day five was compared for each species separately using a randomized block design ANOVA in R v.3.3.2 (R Core Team 2016). Survivorship (number of specimens alive after five days divided by the number of specimens at the start of each trial) was compared for each species in the three temperature treatments with trial (1–5) as a blocking factor.

## Results

### *The marine live bait trade in California*

We identified 404 businesses in coastal counties and 40 in the Sacramento Delta region listed as selling fishing bait and tackle. In telephone calls to each of these, a total of 70 reported selling marine live bait. Of these, the great majority (~ 88%) were located in the San Francisco Bay Area / Sacramento Delta Region (~ 57%), or in metropolitan southern California (~ 31%) (Figure 1).

A total of 24 of these 70 businesses (38.7%) responded to the mail survey. Each responding business carried, on average, 2.45 (SD = 1.47) species of marine live bait. Pileworms, bloodworms, and ghost shrimp were reportedly the most commonly sold species of marine live bait in California with 62.5%, 50%, and 46% of survey respondents selling these



**Figure 1.** Distribution by county of bait shops selling marine live bait in California according to survey responses. Live marine bait was sold in all counties shaded in grey. Numbers inside black circles indicate species sold in that county. Bait shops in shaded counties without black circles reported marine live bait sales but did not provide information on which species they sold.

species, respectively. Lugworms and nuclear worms were both reportedly sold at 17% of the businesses responding to the survey. All of the businesses reported that these species are imported into California from other states or countries. Approximately one third of survey respondents reported selling “grass shrimp,” with all of them listing California as the origin. This species was tentatively identified as the California bay shrimp, *Crangon franciscorum* Stimpson 1865, one of the most common native species of shrimp in central California. Two businesses reported importing other non-marine types of bait (minnows and mudsuckers) from out of state. The location of origin and year of first importation as reported from survey respondents for individual species of marine live bait are shown in Table 1.

#### *Estimates of the number of individuals imported annually*

Our estimates suggest that as of 2009, almost 1,900,000 ghost shrimp were imported annually into California (Table 2). At that time, approximately 600,000 pileworms and nearly 575,000 bloodworms imported from the Northeast coast of the United States were stocked in California bait shops

**Table 1.** Origin and date of first importation as reported by survey respondents of the five most common species imported as marine live bait into California.

| Live bait species                             | Origin               | Year of first importation |
|---|----------------------|---------------------------|
| Bloodworms <i>Glycera dibranchiata</i>        | Maine                | 1972                      |
| Lugworms <i>Perinereis</i> sp.                | South Korea          | 1972                      |
| Nuclear worms <i>Namalycastis</i> sp.         | Vietnam              | 1994                      |
| Pileworms <i>Alitta virens</i>                | Maine, Massachusetts | 1972                      |
| Ghost shrimp <i>Neotrypaea californiensis</i> | Oregon, Washington   | 1978                      |

**Table 2.** Estimates of the number of individuals of five species imported as marine live bait into California annually.

| Live bait species                             | Number of survey respondents (out of 24) | Percentage of survey respondents (out of 24) | Potential number of bait shops (out of 70) | Average number stocked per shop annually [number of respondents providing estimate] (95% C.I.) | Estimated number of individuals imported into CA annually (95% C.I.) |
|---|--|--|--|--|--|
| Bloodworms <i>Glycera dibranchiata</i>        | 12                                       | 50%  | 35   | 16,408 [8] (9,104; 25,458)   | 574,280 (318,640; 891,030)   |
| Lugworms <i>Perinereis</i> sp.                | 4  | 17%  | 12   | 93,000 [1] (NA)  | 1,116,000 (NA)   |
| Nuclear worms <i>Namalycastis</i> sp.         | 4  | 17%  | 12   | 60 [1] (NA)  | 720 (NA)   |
| Pileworms <i>Alitta virens</i>                | 15                                       | 62.5%  | 44   | 13,635 [8] (5,030; 24,934)   | 599,940 (221,320; 1,097,096)   |
| Ghost shrimp <i>Neotrypaea californiensis</i> | 11                                       | 46%  | 32   | 59,164 [7] (8,738; 111,200)  | 1,893,248 (279,616; 3,558,400)                                       |

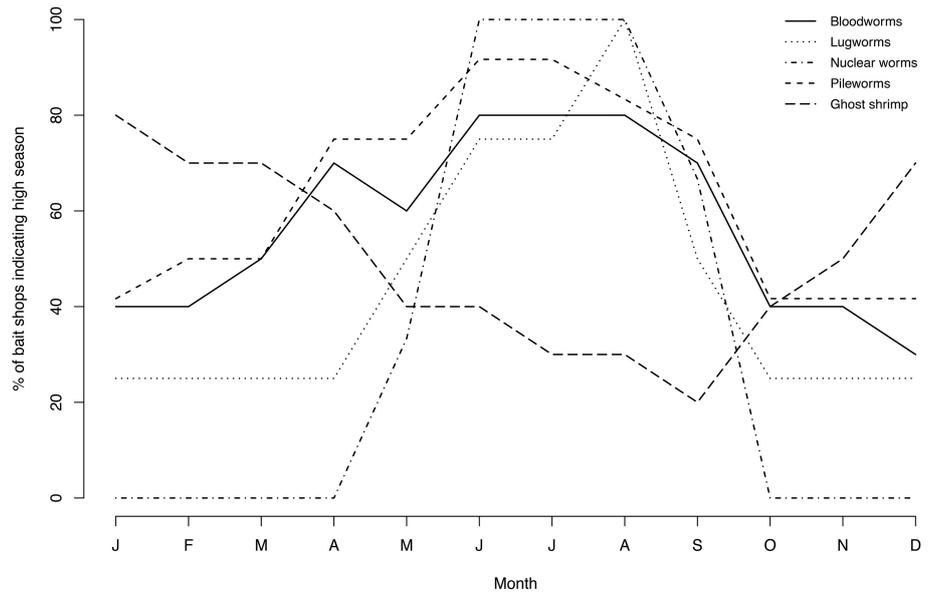
annually. Over one million lugworms were imported annually from Korea, and a few more than 700 nuclear worms were likely imported from Vietnam.

#### *Seasonality, packing materials, and methods of disposal*

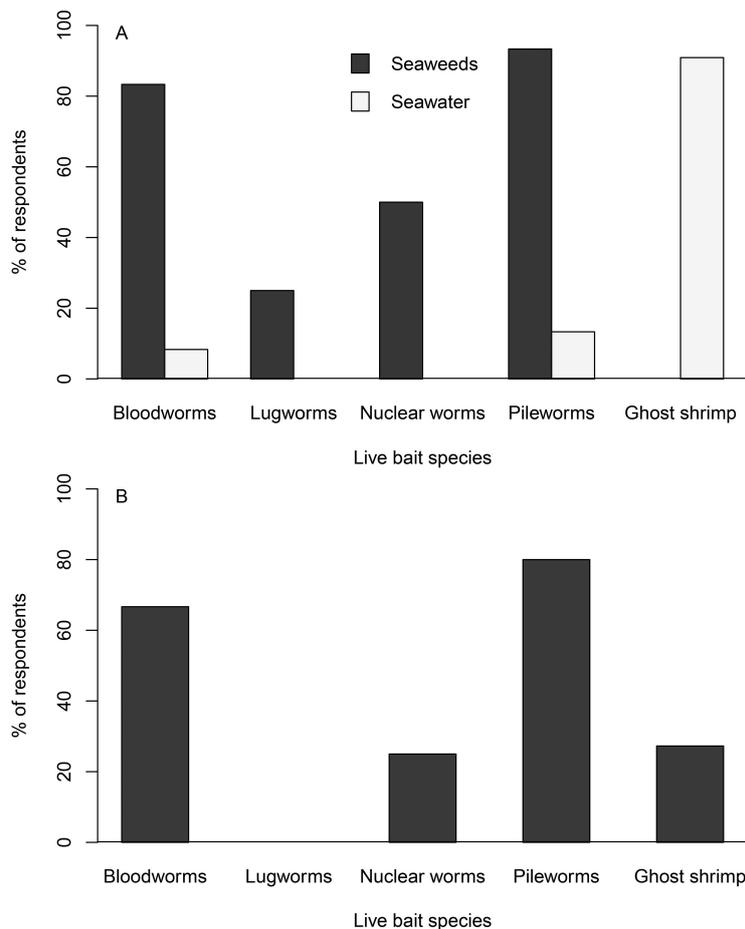
All species of marine live bait were reported to be sold in California year-round. Survey responses suggest that there are different peak sales for baitworms and ghost shrimp (Figure 2). The four baitworm species showed peaks in sales during the summer, while sales of ghost shrimp were greatest during winter months.

Seaweeds (sometimes in combination with newspaper) were reported as the main packing material in live bait shipping, but seawater was also reportedly used as a packing material (Figure 3A). No other types of packing material were reported as used. Bait shops reported that hitchhiker species were present in shipments of nearly all target species of live bait, with the exception of lugworms (Figure 3B). Hitchhikers were reportedly found in the packing materials, or on the bait itself. Survey respondents indicated that seaweeds, sea jellies, clams, snails, worms, barnacles, crabs, shrimp, sand fleas, and finfish were observed in the shipments with the packing materials or on the bait itself.

A variety of methods were used for disposing of packing material (Table 3). A high percentage of the respondents that sell bloodworms (75%) and pileworms (73.3%) provided at least some of the seaweed packing that the worms arrived in to their bait customers. Shops disposed of unsold live bait by throwing it in the trash, toilet, or by using other methods of disposal. A few shops reported that they gave unsold live bait to their customers.



**Figure 2.** Sales seasonality of marine live bait in California. Overall, baitworm sales peak in the summer and ghost shrimp sales peak in the winter.



**Figure 3.** For each species of marine live bait, (A) the percentage of respondents who reported importing that species who answered that either seaweed (sometimes in combination with newspaper) or seawater were used as packing materials, and (B) the percentage of respondents who reported importing that species who observed hitchhikers in shipments of that species. Note that for (A), some respondents indicated that both seaweed and seawater were used as packing materials for a given species, so that the total percentage may add up to greater than 100 (e.g., for pileworms).

**Table 3.** Methods of disposal of seaweed, seawater, and unsold live bait from live bait survey responses.

| Live bait species                                | N  | Disposal of packing materials | Disposal of seawater | Disposal of unsold bait  |
|--|----|-------------------------------|----------------------|--------------------------|
| Bloodworms<br><i>Glycera dibranchiata</i>        | 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)            |
| Lugworms<br><i>Perinereis</i> sp.                | 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<br><i>Namalycastis</i> sp.         | 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)            |
| Pileworms<br><i>Alitta virens</i>                | 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)            |
| Ghost shrimp<br><i>Neotrypaea californiensis</i> | 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

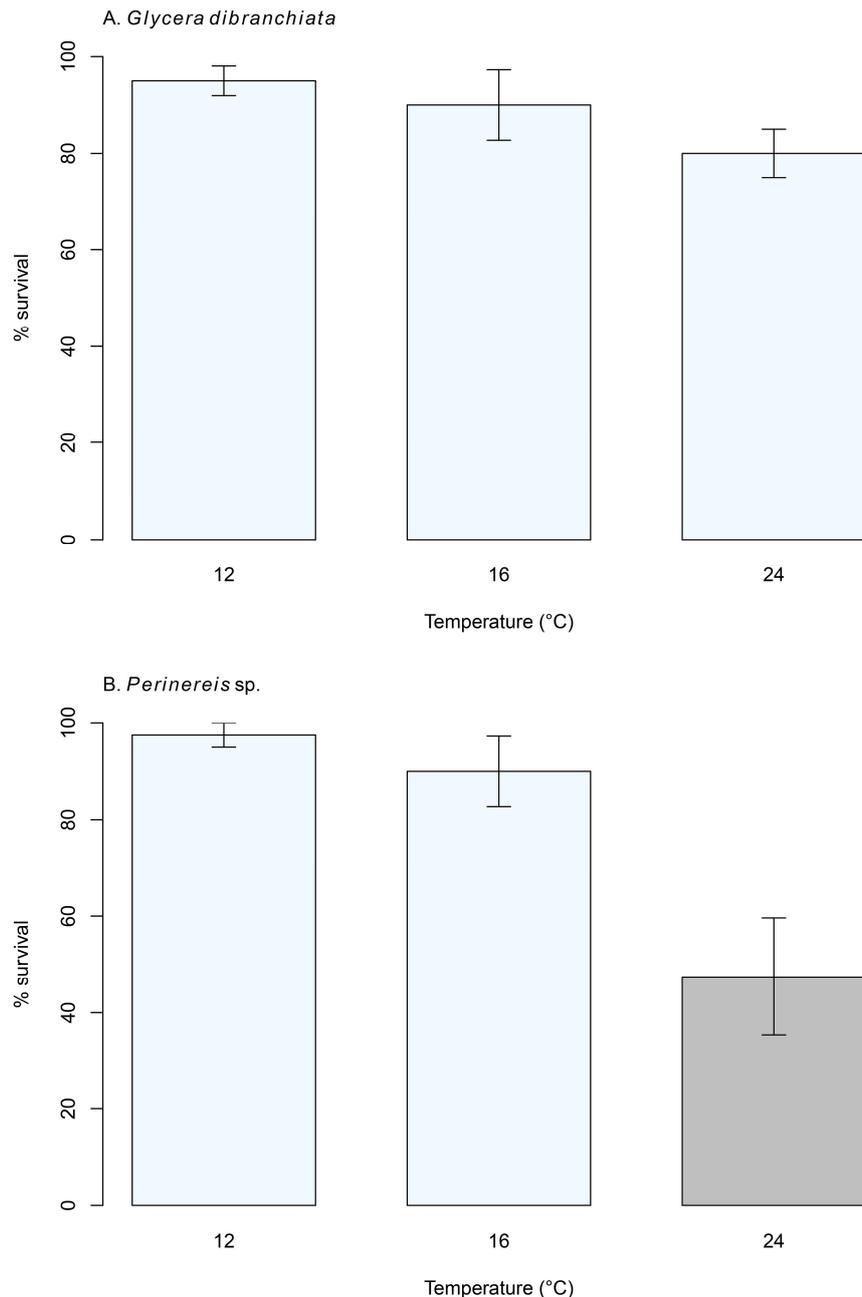
\*\* Survey respondents answered that they dispose ghost shrimp in a garden (3) or feed it to birds (1)

N equals the number of survey respondents that provided information about methods of disposal

Bait shops may have indicated more than one method of disposal (in some cases totals add up to more than N)

### *Survival of non-indigenous live bait in relation to temperature*

Both species tested showed a high percent survival after five days in the two cooler temperature treatments (Figure 4). The percent survival of bloodworms was 95% at 12 °C and 90% at 16 °C. We observed a similar pattern for lugworms, with 97.5% and 90% of the animals surviving at 12 °C and 16 °C, respectively. There was no significant difference in bloodworm survival among the three temperature treatments ( $F_{2,8} = 2.67$ ,  $p = 0.130$ ). For *Perinereis* sp. there was no significant difference in survival between the two cooler treatments, but fewer individuals survived in the highest temperature treatment (ANOVA:  $F_{2,8} = 11.08$ ,  $p = 0.005$ ). No significant difference was found for the blocking factor (trials 1–5) for both species (ANOVA:  $F_{4,8} = 2.00$ ,  $p = 0.188$ , and  $F_{4,8} = 1.14$ ,  $p = 0.403$ , respectively).



**Figure 4.** Average percent survival ( $\pm$  SE) of *G. dibranchiata* (A) and *Perinereis* sp. (B) after exposed to southern California thermal conditions for five days. Gray bars indicate significant difference. No difference in survival was observed among the three temperature treatments for *G. dibranchiata* ( $F_{2,8} = 2.67, p = 0.130$ ). There was a significant difference in survival of *Perinereis* sp. among the treatments ( $F_{2,8} = 11.08, p = 0.005$ ).

## Discussion

This study provides a state-wide estimate of the number of species and number of individuals of marine live bait imported into California annually. We estimated that approximately 575,000 bloodworms, 600,000 pileworms, one million lugworms, and nearly two million ghost shrimp were imported into California annually around 2009 (Table 2). Our results are roughly consistent with those of Cohen et al. (2001), who earlier had estimated that over 600,000 bloodworms and 700,000 pileworms were

imported each year into the San Francisco Bay Area, which we identified as the primary recipient region for imported live bait in the state (Figure 1). We compared the list of bait shops compiled in this study with that in Cohen et al. (2001) and found that the total number of bait shops in the San Francisco Bay Area had not changed between 2001 and 2009 (though there had been substantial turnover in bait shops, i.e. many shops present in 2001 were no longer in existence in 2009, and many shops present in 2009 had not been in existence in 2001). In a report on the live bait trade in California, Cohen (2012) estimated that between 70 and 79 businesses sell marine live bait in California. This estimate is also consistent with our findings.

There are numerous potential sources of error associated with the estimates provided in this study. We may have underestimated the number of businesses selling marine live bait because some of them might not have been listed in online directories under “Fishing bait and tackle” (e.g. liquor stores selling marine live bait). Survey respondents might not have had accurate information or deliberately provided inaccurate information on the number of individuals stocked annually. In addition, not every bait shop identified as selling marine live bait returned a survey, yielding a relatively low sample size. Low return rates for mail surveys could be due to many factors. For example, some bait shop owners may have perceived the survey as a potential threat to their business (if results from the survey might result in an increase in regulations). Some surveys might not have been returned because they were provided only in English, and some bait shops are operated by people whose first language is not English. Furthermore, many business owners (and more generally, citizens) are simply not motivated to participate in surveys. Despite these shortcomings, which are common in mail surveys (Dillman 1991), the response rate to our survey was similar to the response rates obtained in other studies using similar methods (Meronek et al. 1997; Weigle et al. 2005). Finally, not every survey respondent provided estimates of the number of individuals stocked annually (Table 2), reducing sample sizes for estimation of that parameter. In future, one way to corroborate estimates like those we made here from retailer-supplied data is to compare it with information reported by wholesalers on the numbers of individuals imported annually. 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. We suspect that a similar pattern holds for marine live bait, and that the numbers estimated in the present study should be viewed as minimum estimates. An alternative approach to obtaining these data, of course, is for state management agencies to enforce administrative codes requiring a permit to import live bait, and for these agencies to require permit holders to report on species and quantities imported.

Our 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 (Table 1). 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 pattern seems to be driven by the use of ghost shrimp as bait during the sturgeon fishing season in the Sacramento Delta region. If only ghost shrimp data from southern California (where ghost shrimp are used as bait for a diversity of target species, not including sturgeon) are considered, there is no obvious seasonal pattern in sales (data not shown).

In addition to estimates of quantity and a description of sales seasonality of live bait species, our study provided insight into the transport of other NIS that may be imported with the live bait trade: seaweeds and hitchhikers. A few survey respondents indicated that seawater was used in the shipment of bloodworms and pileworms (Figure 3A), which is inconsistent with our observations and the literature (Cohen et al. 2001; Cohen 2012). Typically, these species are shipped packed in seaweed. It is possible that the respondents were not referring to bulk seawater, but to the fact that seaweeds used as packing material are typically damp with seawater. Previous estimates have shown that seaweeds and hitchhikers in bait worm shipments are imported into California in large numbers (Cohen et al. 2001). Seaweeds and several species of marine invertebrates found in live bait packing material may have been introduced via the live bait trade. Cohen et al. (2001) list the seaweed *Codium fragile tomentosoides*, the snails *Littorina littorea* and *L. saxatilis*, and the European green crab *Carcinus maenas* as NIS in the San Francisco Bay Area that were likely introduced via the live bait trade (but see Chang et al. [2011] for discussion of other possible invasion routes for *L. littorea*). *L. saxatilis*, for example, was likely introduced by anglers disposing seaweeds used as packing materials for baitworms (Carlton and Cohen 1998). Our survey shows that hitchhiker species are imported into southern California with most species of live baitworms and with ghost shrimp (Figure 3). Besides free-living hitchhikers, we also found parasites (e.g. *Ione cornuta* and nematodes of the genus *Ascarophis*) infecting imported ghost shrimp (*Neotrypaea californiensis*) sold in bait shops in southern California (B. Passarelli, *unpubl. data*). All of these imported taxa – bait species (baitworms and ghost shrimp), as well as seaweeds and both free-living and parasitic hitchhikers – may possibly be introduced to local marine habitats, where they may potentially become established as NIS. Recent studies have identified and quantified organisms imported with packing algae and suggest that this vector could be a significant source of introduction of NIS (Cohen 2012; Blakeslee et al. 2016; Fowler et al. 2016).

Introduction is only a first step in the invasion process. Many factors, such as physiological limits and community interactions, may affect the likelihood of a species becoming established (Kolar and Lodge 2001; Somero 2005; Kelley 2014). We evaluated one of these: survival after short term exposure to a range of 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 sudden disposal of bait into marine habitats. We found that both imported taxa tested in this fashion (*Glycera dibranchiata* and *Perinereis* sp.) were able to survive at least five days of exposure to the two cooler temperatures (12 and 16 °C). In addition, *Glycera dibranchiata* showed relatively high survival at the highest temperature tested, 24 °C. *Perinereis* sp. showed lower survival at 24 °C in comparison to the two colder treatments. Temperatures above 20 °C are relatively uncommon in southern California marine habitats, and are observed only in shallow habitats such as estuaries and lagoons (NOAA). Those types of habitat represent a small fraction of the total area where recreational fishing occurs. This suggests that temperature probably would not restrict survival of *G. dibranchiata* and *Perinereis* sp. in most marine habitats in southern California. Of course, other factors in addition to short-term thermal tolerance must also be evaluated to determine the ability of non-indigenous bait and associated species to become established in local habitats. These include other organismal traits (e.g. long term survival, reproductive physiology) as well as ecological factors (e.g. availability of appropriate food resources, absence of controlling predators and parasites) (Colautti and MacIsaac 2004).

This study estimated the numbers of individuals imported from donor regions to bait shops. To be introduced, however, propagules must actually be released into the environment. The release of imported bait into the environment is probably common in freshwater systems (Litvak and Mandrak 1993; Keller and Lodge 2007). The freshwater live bait trade in North America has a high economic value (over US \$1 billion annually) and transfers large quantities of live bait species (tens of millions) to areas where they are non-indigenous (Litvak and Mandrak 1993; Meronek et al. 1997). A survey conducted in 1988 with anglers in Ontario showed that 41% of them released unused live baitfish into the freshwater environment. More than half of the anglers were not aware of a regulation prohibiting the release of unused live bait (Litvak and Mandrak 1993). The large numbers of individuals transported via the freshwater live bait trade and the high percentage of freshwater anglers releasing unused live bait suggest that the number of live bait individuals released into freshwater environments may be high. Our data show that a large number of individual bait organisms are imported into California as part of the marine live bait trade. It is likely

that anglers using marine live bait in California also release live bait into the environment (Lau 1995). In addition, responses to our surveys show that disposal of packing materials and unsold live bait sometimes happens in ways that may result in introduction of NIS into local marine habitats (e.g. direct disposal into the ocean by shop owners, or gifting to customers who may then dispose of materials into ocean) (Table 3). Future surveys of anglers are necessary to determine the actual propagule pressure of NIS of marine live bait in California coastal habitats.

The great magnitude and high diversity of organisms transported via the live bait trade suggest that this may be a potent vector for introductions (Cohen 2012; Fowler et al. 2016). Numerous potentially effective management strategies could be implemented to reduce this risk. One obvious strategy would simply be to ban the importation of non-indigenous species of live bait. Less drastically, the state might put into place regulations to limit the acceptable types of packing material used in transporting bait, which would help to reduce the numbers of hitchhiking NIS that might be introduced. Some work suggests that pre-treatment of algal packing materials (e.g., by osmotic shock) might kill hitchhiker species (Blakeslee et al. 2016); the state might require that packing material be treated in some such fashion. The California Department of Fish and Wildlife (CDFW) could also enforce existing rules (California Administrative Code, Title 14, §236(c)(6)(B)–(G)) that prohibit the importation of live bait without a permit; implementing an application process for such permits would allow the state to block importation of particularly risky species. In addition, if CDFW enforced this requirement it could collect information on the species and numbers of organisms imported. Though gathering such data would not reduce the risk of introduction, it would certainly help in making informed decisions about these risks. At the bait shop level, an effective strategy would be prohibiting bait-shop owners from providing the seaweed packing for imported live baitworms to customers and requiring them instead to dispose of the seaweed appropriately. At the very least, using labels that identify marine live bait species and place of origin, educating bait shop owners and anglers about risks posed by the release of these NIS, and providing information about safe methods of disposal of packing materials, seawater, and unwanted bait could help to minimize risks of introductions.

The data reported here do not allow us to generate a comprehensive estimate of the risk of introduction and establishment of marine live bait species into California waters. They do, however, provide rare estimates of importation patterns of marine live bait species, as well as simple physiological data suggesting that thermal stress is unlikely to limit establishment of imported bait species in typical temperature conditions found in southern California marine habitats. These types of data, in combination with other

approaches (Cohen et al. 2007; Kilian et al. 2012; Grosholz et al. 2015; Blakeslee et al. 2016; Fowler et al. 2016), can be used to further assess and manage risks of the marine live bait trade in California as a potential pathway for the introduction of NIS.

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### Supplementary material

The following supplementary material is available for this article:

**Appendix 1.** Live marine bait survey questionnaire.

This material is available as part of online article from:

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