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THE INTRODUCED SABELLID POLYCHAETE *TEREBRASABELLA HETEROUNCINATA* IN CALIFORNIA: TRANSMISSION, METHODS OF CONTROL AND SURVEY FOR PRESENCE IN NATIVE GASTROPOD POPULATIONS

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ABSTRACT The sabellid polychaete *Terebrasabella heterouncinata* (Fitzhugh & Rouse 1999) has a unique life history in which larvae settle on the edge of gastropod shells and rely on shell deposition to create a tube with an opening to the exterior. This worm was accidentally imported to California, USA on abalone from South Africa in the 1980s and spread with abalone shipments to most culture facilities and some public aquaria throughout the state. Its ability to infest California's native gastropods has sparked concern regarding potential establishment in intertidal habitats adjacent to facilities that held sabellid-positive abalone. We examined the ability of *T. heterouncinata* to transmit between individual turban snails, *Tegula funebralis*. We found that transmission between *T. funebralis* did occur, but at a significantly slower rate than that between red abalone *Haliotis rufescens*. During 2002 to 2006 native gastropods (turban snails and limpets) were collected at most sabellid-exposed sites and no *T. heterouncinata* were detected; it thus appears that this species has not become established in California. Freshwater exposure was examined as a method to kill *T. heterouncinata* in shell fragments that may remain after abalone are removed from production or display units. Freshwater immersion for up to 8 hours but not 16 or 32 h resulted in survival of adults and/or larvae resident in brood chambers. In a similar study, motile *T. heterouncinata* larvae were found to survive up to 32 sec of freshwater exposure, whereas none survived a 64-sec exposure. These data can be used by abalone culture and display facilities to establish reliable sanitization procedures to prevent *T. heterouncinata* transmission or reinfestation.

KEY WORDS: *Terebrasabella heterouncinata*, sabellid polychaete, abalone, *Haliotis*, *Tegula funebralis*, gastropod, aquaculture

INTRODUCTION

In 1990 a California abalone (*Haliotis* spp.) farm first experienced what became an epidemic of animals with domed, brittle shells and deformed respiratory pores (Oakes & Fields 1996). The condition was linked to the presence of tiny (2-mm) sabellid polychaetes infesting the abalone shell. The polychaete was found to be a previously undescribed species, inadvertently imported during the mid1980s with South African abalone that were brought in for research purposes (Kuris & Culver 1999).

This new species of fan worm, *Terebrasabella heterouncinata* (Sabellidae), is unique in exploiting gastropod shell deposition to create a protective tube (Fitzhugh & Rouse 1999). Motile benthic larvae emerge from a brood chamber in the adult tube and may settle on the same shell or migrate to other susceptible gastropods nearby. The settling larva secretes a mucus sheath that the abalone covers with shell material, resulting in a tube with a sealed basal end and an anterior end open to the outside of the shell (Fig. 1A). The larva metamorphoses into an adult with a branchial feeding crown (Fig. 1B). Finley et al. (2001) demonstrated that these sabellids, which are hermaphroditic, are capable of self-fertilization and, on farmed red abalone hosts, can develop and reproduce at temperatures spanning those encountered in California (11.2°C, 15.6°C, and 20.9°C). Generation time was negatively correlated with water temperature.

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Although light infestations do not result in changes in shell morphology or structure, dramatic changes occur with heavy infestations of hundreds of worms on a single shell. Heavily infested abalone reduce or cease production of the prismatic layer of the shell, whereas continuing nacreous layer secretion, resulting in vertical shell growth (Kuris & Culver 1999). Over several months of culture, farmed abalone with heavy infestations develop brittle shells that are domed in shape with deformed or absent respiratory pores (Fig. 2).

The dependence of abalone production facilities on a small number of abalone seed producers resulted in the spread of *T. heterouncinata* infestations to nearly all of California's abalone culture facilities by 1995. The poor price obtained for abalone with stunted, deformed shells and the destruction of large numbers of submarket-size animals was directly responsible for the failure of several farms, whereas others suffered severe financial hardship from which they eventually recovered. Infestations were also discovered at public aquaria and educational facilities that purchased infested animals (Moore & Robbins, unpublished data).

Examination of nonhaliotid gastropod cohabitants in abalone production systems indicated that common intertidal gastropods such as turban snails (*Tegula* spp.) and limpets (*Lottia* spp.) were susceptible to infestation, at least under conditions of intense exposure (Kuris & Culver 1999). Culver and Kuris (2004) provided more detail on the relative susceptibility of various California gastropods when exposed to infested abalone. However, the life history traits of

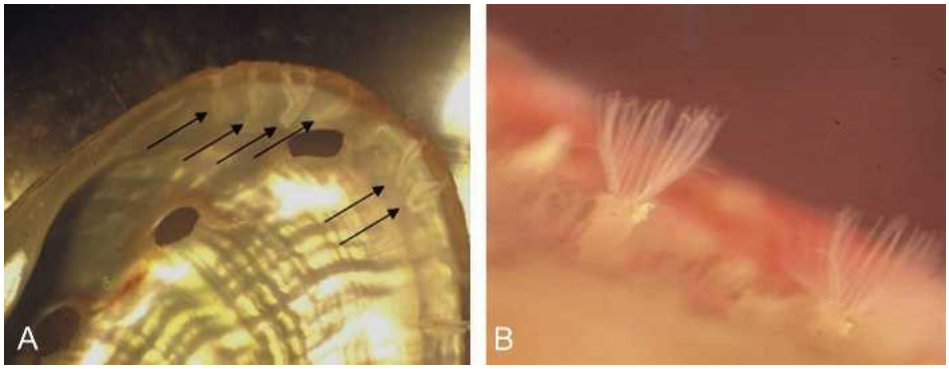

Figure 1. ***Terebrasabella heterouncinata*** **infesting farmed red abalone** ***Haliotis rufescens*****. A: Ventral view of lightly-infested juvenile** ***H. rufescens*** **shell with soft tissues removed. Arrows point to tubes of recently settled** ***T. heterouncinata*****. B: Crowns of adult** ***T. heterouncinata*** **emerging from burrows on the dorsal surface of** ***H. rufescens*** **shell.**

T. heterouncinata on nonhaliotid hosts and the ability for horizontal transmission in the absence of abalone has not been demonstrated. Almost nothing is known about the infestation dynamics on susceptible nonhaliotid gastropods (e.g., limpets and turban snails), the species that are likely to sustain and spread infestations because of they can occur at very high densities in rocky intertidal and nearshore subtidal habitats statewide.

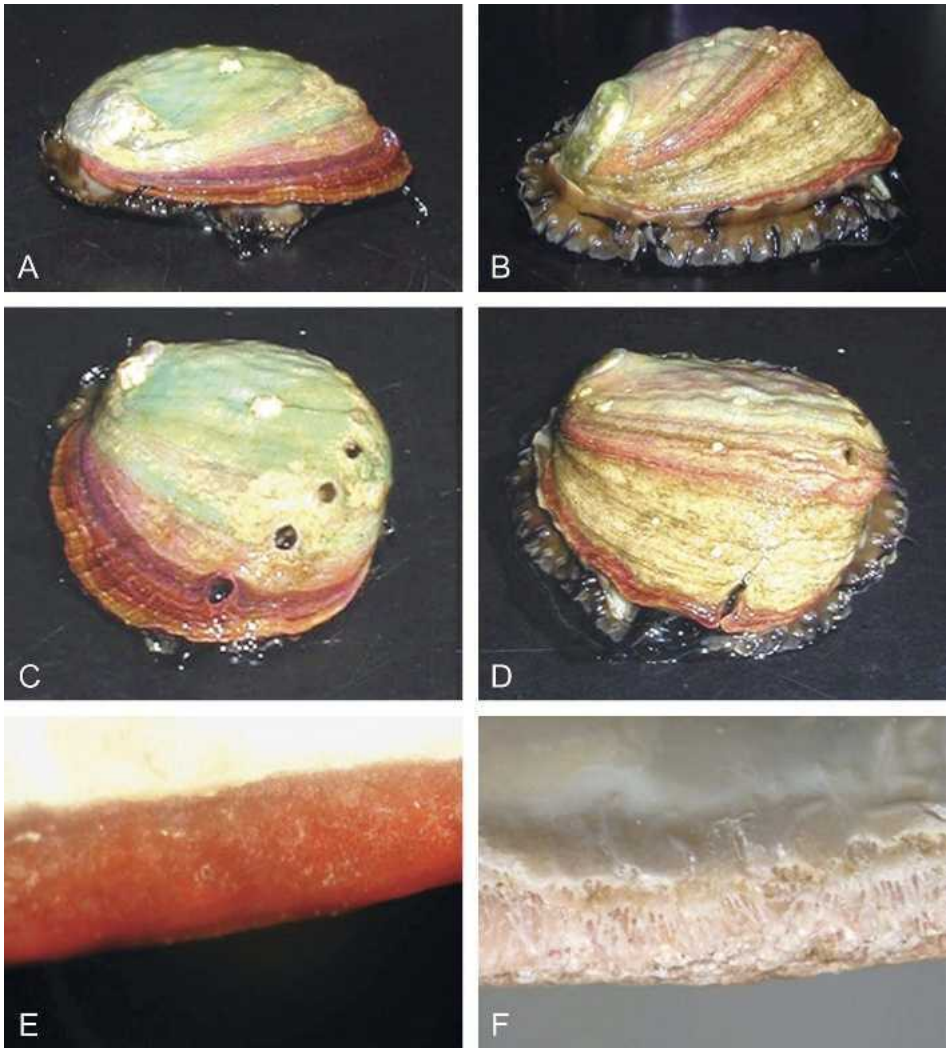

Figure 2. Effect of ***T. heterouncinata*** **infestation on farmed red abalone** ***H. rufescens*****. A and C: Side and front views of an uninfested abalone. B and D: Similar views of a heavily infested abalone, showing vertical shell growth and deformed respiratory pores. E: Shell margin of uninfested abalone, showing a uniform prismatic layer deposition. F: Shell margin of an abalone heavily infested with** ***T. heterouncinata*****, showing tube entrances and a lack of prismatic layer deposition. For E and F the ventral surface of the shell is above.**

Outside land-based farms, *T. heterouncinata* entered the intertidal environment as dislodged larvae in the effluent stream and *via* the practice of rinsing empty shells and shell debris down discharge outfalls. As feared, *T. heterouncinata* -infested intertidal gastropods were discovered outside one infested facility near Cayucos in 1996 (Culver & Kuris 2000). Surveys indicated the infestation was limited to the immediate discharge area (<100 M of shoreline). An extensive eradication effort was conducted, based on the concept of host-density threshold, resulting in the removal of 1.6 million gastropods (primarily turban snails, *Tegula funebralis*) in 1997 (Culver & Kuris 2000). Most other exposed sites have not been systematically surveyed for the presence of *T. heterouncinata*, and the threat of significant harm to members of California's complex benthic species assemblages has been of great concern to the marine conservation community (Kuris & Culver 1999, Culver & Kuris 2000). Although the hosts and all life stages of this worm are benthic, spread of a primary infestation to remote sites could occur by rafting of infested hosts on drifting macroalgae or possibly by floating near the water surface *via* a mucus thread held by surface tension (Ruck & Cook 1998).

In 1997, in parallel with the efforts being conducted at the Cayucos site, the California Department of Fish and Game developed a Sabellid Eradication Program to eliminate the pest from the state, including site-specific risk assessments and renewal of operating permits being contingent on implementation of cleanup plans. This effort has been successful in eliminating *T. heterouncinata* from most, if not all, infested facilities. The severe economic hardship resulting from *T. heterouncinata* infestation prompted a number of studies to develop methods to kill the sabellid without killing abalone, including heat (Leighton 1998), microencapsulated pesticides (Shields et al. 1998), coating the shell surface with wax, and biological control (Kuris & Culver 1999), but none have proven effective in the farm environment. Ultimately, proper hygiene and rigorous avoidance of mixing infested and uninfested stocks has been successful at eliminating *T. heterouncinata* from abalone culture and display facilities. Two key issues are preventing transfer of *T. heterouncinata* larvae as workers move from tank to tank, and preventing the retention of larvae and/or adult stages in shell fragments when one batch of abalone is moved out of a tank and another is moved in. This is particularly problematic because heavily-infested shell is unusually brittle, and many *T. heterouncinata* could be present in tiny (i.e., several mm in area) shell fragments that are difficult to remove from large-scale production tanks, barrels or raceways. Culver et al. (1997) conducted preliminary studies indicating that larvae can die within seconds of freshwater exposure, whereas exposure of infested shell for two hours killed adults although larvae within the brood chamber remained viable. These data helped the industry largely overcome the sabellid problem. However, at a number of facilities the eradication process was slower than anticipated and *T. heterouncinata* reappeared at other facilities where complete eradication was believed to have been accomplished. In this study, we formally address several issues surrounding *T. heterouncinata* transmission and control: (1) the extent of transmission among turban snails; (2) the distribution, if any, in natural populations of turban snails and limpets, (3) the freshwater exposures needed to control adults and larvae.

MATERIALS AND METHODS

Experimental Animals

Red abalone *Haliotis rufescens* were donated by The Abalone Farm, Inc., (Cayucos, CA, USA) and turban snails *Tegula funebralis* were collected locally at the Bodega Marine Laboratory, Bodega Bay, CA, USA. All experiments involving *T. heterouncinata* were conducted in the California Department of Fish and Game's Pathogen Containment Facility at the Bodega Marine Laboratory. All effluent from the Pathogen Containment Facility is chlorinated and de-chlorinated prior to release. Additionally, effluent from the wet table holding tanks containing *T. heterouncinata*-infested animals was passed through a 75-μm filter to capture sabellid larvae. The source of *T. heterouncinata* included infested red abalone from various farms and display facilities in California collected from the mid1990s through 2002.

Transmission of T. heterouncinata *between* Tegula funebralis

Fifty uninfested turban snails *T. funebralis* and fifty uninfested red abalone *H. rufescens* were individually tagged by gluing numeric plastic tags to the outside of each shell. All were then commingled with 15 red abalone moderately- to heavily-infested with *T. heterouncinata* in a 4-L container with flowing seawater at 17.8°C, a temperature conducive to reproduction of this sabellid (Finley et al. 2001). Each day, the exposed shell margins on the previously uninfested animals were examined and individuals with one to four new sabellid tubes were placed in a separate container to prevent additional recruitment. The required number of 25 newly infested individuals of each species (see later) was achieved on the 11th day of exposure, and the experiment was initiated the following day. The newly infested animals were divided into five replicate ''donor'' groups of five individuals each and were dispersed into to five new 4-L containers, separately for each species. The experiment was initiated with the addition of five tagged, uninfested conspecifics (as ''recipients'') to each of the replicate tanks. The presence of newly settled *T. heterouncinata* on all donors and all recipients was recorded weekly. Abalone died throughout the experiment because of the presence of an unrelated disease, withering syndrome, and the experiment ended when all of the five donor abalone in one replicate tank died. Rates of *T. heterouncinata* transmission for each species were compared by testing for differences in slope (Zar 1984) using tank means (Fig. 3) for recipients from the week before the first appearance of a sabellid tube through the first week with no change in settlement. For this analysis the dependent variable was defined as the tank mean value for the cumulative number of recipients that acquired at least one sabellid tube, which was considered to be the best indicator of the ability of *T. heterouncinata* to reproduce and spread to members of the same host species. To examine differences in intensity of infestation between the two species, we also calculated the mean number of *T. heterouncinata* that accumulated on donors and recipients of each species over the study period, on a per-individual basis to account for abalone mortalities. Differences between the four groups were examined with an ANOVA by Ranks test with Student-Newman-Keuls multiple comparisons. The mean temperature over the study period was 18.3°C.

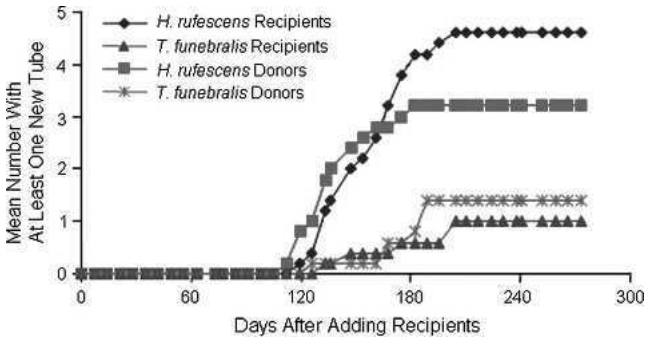

Figure 3. Transmission of *T. heterouncinata* between *H. rufescens* individuals and between *T. funebralis* individuals. Ordinate axis shows the cumulative mean number (for five replicate tanks) of individual donors or recipients of each species that acquired at least one new sabellid at the time periods shown.

Survey of Native Gastropod Populations for the Presence of T. heterouncinata

Tegula spp. (primarily *T. funebralis*) and several species of limpets (primarily *Lottia pelta, L. digitalis, L. asmi. L. limatula,* and *Maclintockia scabra*) were collected at intertidal locations known to have been, or suspected to have been, exposed to *T. heterouncinata* through release from adjacent abalone culture, holding or display facilities. Collections (Table 1) were made from 2003 to 2005 except for the South Carmel (2002), Port Hueneme (2006), and Carlsbad Lagoon (2006) sites. Sampling procedures were tailored to each site based on topography, population density and distribution, and time limitations governed by tidal cycles. The risk or extent of release was characterized as low-to-absent, low, moderate or high based subjectively on our knowledge of the number of, and period of time for which, infested abalone were present. The receiving environment susceptibility was subjectively characterized as low, moderate or high based on the proximity and density of susceptible hosts. At locations where animals were abundant, 60 individuals of each species were collected from discrete geographical units in the vicinity of the discharge or exposure zone and additional samples of 60 were taken from populations up to approximately 100 M to the north and south. A sample size of 60 allows for detection with 95% confidence if the prevalence is at least 5% (USFWS and AFS-FHS, 2004). All collected animals were examined for the presence of *T. heterouncinata* tubes with the aid of a dissecting microscope.

Time of Freshwater Exposure to Kill All T. heterouncinata *Life Stages*

Six freshly shucked *H. rufescens* shells on which a minimum of four live *T. heterouncinata* were present (identified by emerging branchial crowns) were immersed in freshwater (Bodega Marine Laboratory well water, 4 ppt salinity) for each of the following time periods: 0, 2, 4, 8, 16, or 32 h. After immersion, shells were returned to seawater and commingled with 10 live, uninfested *H. rufescens* for four months at 18°C. The original shells were examined for the presence of emerging sabellid crowns (as an indicator of survival) at 24 h, 48 h, and monthly thereafter. The 10 untreated "sentinel" *H. rufescens* were examined monthly for the presence of sabellid tubes, which would indicate survival of larval stages within brood chambers in the treated shell.

Time of Freshwater Exposure to Kill Motile T. heterouncinata *Larvae*

Red abalone infested with *T. heterouncinata* were shucked and the shells were broken apart in a dish of seawater to release the sabellid larvae present in brood chambers. Motile larvae were transferred with a Pasteur pipette to individual wells of a 12-well cell culture tray filled with seawater. The seawater was drawn off by pipette without disturbing or removing the larva and 1-mL of Bodega Marine Laboratory well water was pipetted into the well and left there for 0, 8, 16, 32, or 64 sec, followed immediately by flooding with 2.5 mL of seawater. Immediately afterward, most of the water in the well was drawn off and then refilled with more seawater to completely restore salinity. For the 0-sec time point replicates, seawater was drawn off and immediately replaced with new seawater. Larvae were observed immediately after restoring salinity, and at 20 min, 1 and 2 h later. Those capable of physical movement along the bottom of the well were scored as survivors. Ten independent trials with newly isolated larvae were conducted for each exposure period.

RESULTS

Transmission of T. heterouncinata *between* Tegula funebralis

At the start of the experiment, there was a mean of 1.84 ± 0.04 (mean ± se) *T. heterouncinata* per individual among the *H. rufescens* donors and 1.76 ± 0.07 per individual among the *T. funebralis* donors; this difference was not significant (*t*-test; $P > 0.05$). The proportions of individuals having 1, 2, 3, or 4 new tubes also did not differ between the species (χ^2 test, $P > 0.05$). The first newly settled *T. heterouncinata* was observed on an *H. rufescens* donor on day 112 (Fig. 3). The first newly settled sabellid on an *H. rufescens* recipient was observed on day 119. On day 126 the first newly settled sabellid on a *T. funebralis* donor was observed and the first newly settled sabellid on a *T. funebralis* recipient was observed on day 133.

Best-fit lines for differences in slope were used to compare the rates of transmission for *H. rufescens* and *T. funebralis*. The equations were: *H. rufescens*: $y = -6.181 + 0.0549 \times$ ($n = 14$, $R^2 = 0.979$); *T. funebralis*: $y = -1.087 + 0.00936 \times$ ($n = 12$, $R^2 = 0.878$). The slopes are significantly different ($P < 0.001$) indicating a higher rate of transmission between *H. rufescens*.

At the end of the experiment, *H. rufescens* donors and recipients had more new *T. heterouncinata* than *T. funebralis* donors and recipients (ANOVA By Ranks, $P = 0.002$; Student-Newman-Kuels multiple comparison, $P < 0.05$ for each comparison). Figure 4 indicates the intensities of infestation among the individuals present at each time point. The experiment ended when *H. rufescens* mortalities reached a level such that in one replicate tank all five donor abalone had died. On this day, *H. rufescens* mortalities for donors reached 19 total; among *H. rufescens* recipients 5 died, and for all *T. funebralis* only two individuals died, both of which were donors.

Survey of Native Gastropod Populations for the Presence of T. heterouncinata

Native gastropods were collected from 23 of the 25 sites where release of *T. heterouncinata* to the environment was known or suspected (Table 1). The number of *Tegula* spp. and/or limpets (mostly *Lottia* spp. and *McClintockia scabra*) collected varied widely between sites, reflecting variation in

TABLE 1.

Collections of *Tegula* spp and various limpets from intertidal locations (north to south) adjacent to facilities that held or may have held sabellid-infested abalone.

Site #	Location	*Tegula* spp*	Limpets*	Risk or Extent of Release	Source of Release	Receiving Environment Susceptibility	Receiving Environment Description
1	Crescent City	125	124	Low	Suspended cage culture	Moderate	Harbor riprap
2	Trinidad Harbor	144	52	Low	Outfall from partial recirculation system	Moderate	Beach with boulders/tidepools
3	Albion	0	58	Low	Suspended cage culture	Low	Estuary with boulders
4	Point Arena	263	255	Low	Outfall	High	Rocky intertidal
5	Bodega Bay	695	90	Low to absent	Outfall	High	Rocky intertidal
6	Estero Americano	110	144	Moderate	Outfall	Low	Beach & estuary with boulders
8	Tomales Bay (A)	2	136	Moderate	Outfall	Low	Tidal flat with boulders
7	Tomales Bay (B)	0	0	Low	Suspended cage culture	Low	Sandy beach
9	Pillar Point Harbor	72	198	Low	Suspended cage culture	Moderate	Harbor riprap & beach with boulders
10	Davenport (A)	261	279	Low	Outfall	High	Rocky intertidal
11	Davenport (B)	135	69	Moderate	Outfall	High	Rocky intertidal
12	Monterey Harbor (A)	43	2	Low	Suspended cage culture	Low	Pier pilings in sand
13	Monterey Harbor (B)	459	62	Moderate	Suspended cage culture	Moderate	Rocky intertidal
14	South Carmel	69	0	Low	Outfall	High	Rocky intertidal
16	Cayucos (A)	583	0	Moderate	Outfall	High	Rocky intertidal
15	Cayucos (B)	1872	15	High	Outfall	High	Rocky intertidal
17	Goleta	93	131	High	Outfall	Moderate	Rocky intertidal
18	Santa Barbara Channel	not done	not done	Moderate	Suspended cage culture	Low	Offshore sand
19	Port Hueneme	129	48	Low	Outfall	Low	Harbor riprap
20	Oxnard	not done	not done	Low	Outfall	Low	Power plant forebay
21	Santa Monica	0	60	Low to absent	Outfall to sand basin	Low	Pier pilings in sand
22	Redondo Beach	0	0	Low to absent	Outfall	Low	Offshore sand
23	Terminal Island	0	20	Low	Outfall	Low	Pier pilings and cement
24	Carlsbad Lagoon	63	20	Low	Suspended cage culture	Low	Lagoon with high sedimentation
25	La Jolla	120	0	Low	Outfall	Low	Beach with boulders & tidepools
	Total Animals	5238	1763				

* Tegula refers to *T. funebralis*, *T. brunnea* or *T. montereyensis*.
** Limpet refers to all species of limpets encountered.

population density. No *T. heterouncinata* were detected in any gastropods from any of the locations sampled. At the Davenport (B) site, effluent from the farm was released into a sea cave in a region with highly exposed, nearly vertical relief that was extremely difficult to access. Limpets were sampled 800 M north of the source and *T. funebralis* were sampled 500 M south of the source. Cayucos (B) is the site of the sabellid eradication reported by Culver and Kuris (2000) and sampling there used the transect locations reported in their study. The Cayucos (A) site is a separate outfall for other portions of the same farm. The Santa Barbara Channel (offshore) site was not sampled because of its lack of habitat for susceptible gastropods. All abalone and equipment at this location were removed by the operator and/or a subsequent lessee. A second site that was not sampled was a research facility in Oxnard. Discharge from this facility enters a concrete sump that leads to the forebay of a power generating facility. Periodic heat treatments (41°C, 2 h) kill all gastropods in the system. A public aquarium in San Pedro was found to harbor abalone infested with *T. heterouncinata*. This facility has a partially-recirculating seawater system in which effluent is periodically drained into subsurface sand, and release of *T. heterouncinata* is not possible. The facility is, therefore, not included in Table 1. All infested gastropods at the facility were destroyed.

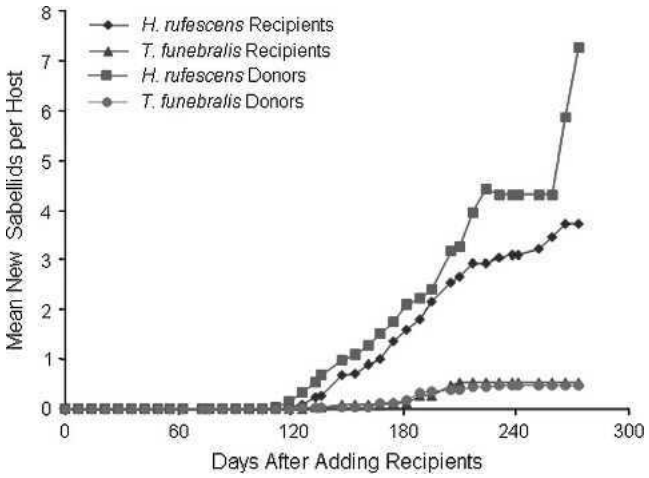

Figure 4. Intensities of *T. heterouncinata* infestations on *H. rufescens* and *T. funebralis* during the transmission experiment. For the donors, only newly acquired *T. heterouncinata* individuals are shown.

Time of Freshwater Exposure to Kill All T. heterouncinata *Life Stages*

Survival of Adults in Treated Shells

On examination at 24 and 48 h posttreatment, sabellid branchial crown emergence was observed in all six pieces of infested abalone shell exposed to freshwater for 0 and 1 h, in three of the six shells exposed for 2 h, and in none of the shells exposed for 4, 8, 16, and 32 h (Table 2). However, re-examination of the shells at one month posttreatment revealed the presence of crowns in five of the six shells in the 4-h treatment in addition to all six shells in each of the 0-, 1-, and 2-h treatments. For the 8-h treatment no crowns were observed at the one-month observation yet one was observed two-months posttreatment.

Transmission to Untreated Abalone

At one month posttreatment, all 10 of the live *H. rufescens* commingled with the shells immersed in freshwater for 0, 1, or 2 h were positive for *T. heterouncinata* tubes. Over four months, recipients commingled with shell treated for up to 8 h became infested whereas those commingled with shell treated for 16 or 32 h did not (Table 3).

Time of Freshwater Exposure to Kill Motile T. heterouncinata *Larvae*

Immersion of *T. heterouncinata* larvae in freshwater for 8 sec caused no change in their behavior relative to unexposed controls, and motility (as an indicator of survival) was observed in all individuals immediately after and 20 min, 1 h, and 2 h after treatment (Table 4). Immersion in freshwater for 16 and 32 sec caused a dramatic reduction in survival, with two larvae being motile two hours after either treatment. No motility was seen at any time point following immersion in freshwater for 64 sec.

DISCUSSION

Transmission of T. heterouncinata *between* Tegula funebralis

Our studies showed that *Terebrasabella heterouncinata* can spread between members of a native California nonhaliotid gastropod species. Newly settled *T. heterouncinata* larvae on *T. funebralis* metamorphosed, developed, and produced viable larvae, some of which settled on the shell of the same host and others of which migrated to other *T. funebralis,* upon which settlement and development to the adult stage occurred. Ruck and Cook (1998) noted that in its native habitat, *T. heterouncinata* infested multiple gastropods and was most common in gregarious lower intertidal species, and they suggested that larval transfer between species may occur when susceptible species co-occur. The previous studies of Kuris and Culver (1999) and Culver and Kuris (2004) demonstrated that a wide variety of native California archeogastropods were susceptible to infestation when commingled with heavily infested red abalone. These findings prompted great concern regarding the potential for *T. heterouncinata* to become established in gastropod populations throughout the state, and potentially beyond. Our findings support the need for the efforts that were undertaken at the Cayucos site (Culver & Kuris 2000).

TABLE 2.

Survival of *Terebrasabella heterouncinata* exposed to freshwater.

	Time of Examination PostExposure						
Exposure Period	Prior	24 h	48 h	1 mo	2 mo	3 mo	4 mo
0 h	6	6	6	6	6	6	6
1 h	6	6	6	6	—	—	—
2 h	6	3	3	6	—	—	—
4 h	6	0	0	5	5	5	5
8 h	6	0	0	0	1	0	0
16 h	6	0	0	0	0	0	0
32 h	6	0	0	0	0	0	0

Note: Six replicate shell fragments were immersed in freshwater for each exposure period. The number of fragments with at least one emergent sabellid crown is shown.
—, not done.

TABLE 3.

Transmission of *Terebrasabella heterouncinata* larvae from infested abalone shell fragments exposed to freshwater.

	Time of Examination after Commingling with Treated Shell				
Exposure Period	Prior	1 mo	2 mo	3 mo	4 mo
0 h	0	10	10	10	10
1 h	0	10	—	—	—
2 h	0	10	—	—	—
4 h	0	10	10	10	10
8 h	0	1	2	2	2
16 h	0	0	0	0	0
32 h	0	0	0	0	0

—, not done.
Note: Shown is the number of abalone (out of ten) that became infested with at least one sabellid after commingling with treated shell for the time periods specified.

TABLE 4.

Survival of sabellid larvae exposed to freshwater. Ten independent trials with individual larvae were conducted for each treatment period. The number that were observed to be motile is shown.

	Time of Examination Posttreatment				
Exposure Period	Prior	Immediately Following	20 min	1 h	2 h
0 sec	10	10	10	10	10
8 sec	10	10	10	10	10
16 sec	10	6	5	5	2
32 sec	10	2	3	3	2
64 sec	10	0	0	0	0

We found that both the rate of transmission to uninfested animals and the intensities of infestation that developed over the study period were higher among *H. rufescens* than among *T. funebralis,* indicating that the former was a more suitable host species for *T. heterouncinata*. Mechanisms that could account for the differing rates of transmission and intensity of infestation between *H. rufescens* and *T. funebralis* remain unclear. Culver and Kuris (2004) reported similar infestation rates for these species and several species of limpets (relative to more resistant gastropods) when exposed to heavily infested abalone shell. They identified a number of factors that may affect *T. heterouncinata* settlement success. *H. rufescens* and *T. funebralis* both share a lack of specific attributes that were associated with resistance to *T. heterouncinata*, such as the highly polished shell surface of olive shells (*Olivella biplicata*) and the shell cleaning behavior of the blue top snail (*Calliostoma ligatum*). Our results may differ from those of Culver and Kuris (2004) because our recipients were exposed to much lower intensities of infestation. Despite differences in infestation rates, the amount of time that it took newly settled *T. heterouncinata* to reach reproductive maturity was similar between the host species, suggesting that the relatively poor settlement on *T. funebralis* could be because of the ability of the host to prevent settlement rather than providing an intrinsically inferior habitat.

Within each species, the number of *T. heterouncinata* that settled on recipient animals was similar to the number that settled on the donors, indicating an approximately equal rate of migration from the individual harboring the sabellid parent, and settlement on that individual. Larval behavior has not been studied, e.g., whether larvae that settle on the parent's host shell migrate off the shell and return or settle directly without migration. Finley et al. (2001) reported that the time at which 50% of newly settled *T. heterouncinata* produced larvae was 111 days at 20.9°C, 165 days at 15.6°C, and 298 days at 11.2°C. Our findings of the first newly settled *T. heterouncinata* on abalone donors at day 112 and abalone recipients at day 119 at a temperature of 18.3°C agree well with these data.

Survey of Native Gastropod Populations for the Presence of T. heterouncinata

Concern over the potential establishment of *T. heterouncinata* in gastropod populations adjacent to infested facilities prompted us to conduct a statewide survey at all sites known or suspected to be exposed to this sabellid. This was not the first sampling of this type; since the mid1990s both the California Department of Fish and Game and researchers at the University of California, Santa Barbara conducted numerous samplings at higher-risk locations to determine whether the sabellid had become established (unpublished data). To our knowledge no *T. heterouncinata* were found, with the exception of the Cayucos site. Again excepting the Cayucos site, these investigations were limited in scope, yet they provided some assurance that *T. heterouncinata* was not becoming widely established. In every case in which sample sizes in Table 1 are low (or zero), this reflects low animal densities and correspondingly low risk of sustainable sabellid infestation. Additional sampling is warranted at high-risk sites for at least several years in the future. We also anticipate learning of additional *T. heterouncinata*-exposed sites that will need to be monitored.

Time of Freshwater Exposure to Kill All T. heterouncinata *Life Stages*

The large impact that *T. heterouncinata* infestations had on California abalone farm profitability and indeed survival led to rapid development of sanitary control measures. Treating production or holding units, to prevent potential carryover of *T. heterouncinata* that may be present on shell fragments, was identified as a critical control point, and both drying and filling units with freshwater were identified as potential control methods. Drying is effective in situations where production units can be exposed to direct sunlight, but at most facilities they are held under shade and can be difficult to completely drain. Generally freshwater treatment should provide a more reliable management technique. Culver et al. (1997) reported that preliminary studies indicated a period of more than two hours is required to kill all life stages of *T. heterouncinata* within infested shell. Our studies confirmed this and extend the period at which *T. heterouncinata* can survive to eight hours of freshwater immersion. In these studies we deliberately used well water with a salinity of 4 ppt, rather than water with a lower ionic content, so that our studies would be directly applicable to farm environments. It is possible that this elevated tonicity provided protection, or delayed onset of lethal changes, compared with use of more pure water.

In the eight-hour freshwater immersion treatment, an intact sabellid crown was observed on one of six shell treated fragments two months after treatment. Although in the absence of gastropod hosts *T. heterouncinata* larvae will settle on other surfaces, they die in the absence of shell deposition (unpublished observations). Therefore this individual represented an adult or subadult that survived the exposure, although it was not observed at three and four months postexposure, possibly because it died or did not emerge from its tube during the observation period. Also in the eight-hour treatment, one newly settled larva was observed among previously uninfested abalone commingled with the treated shell at one month post treatment, and two were observed at two, three, and four months posttreatment respectively. We hypothesize that the intensely hypo-osmotic environment during immersion in freshwater may result in swelling of the adult individuals within tubes, creating a plug that protects the developing stages within the brood chamber from freshwater exposure.

Culver et al. (1997) recommended treating production units with freshwater for 48 h or for 24 h with the addition of bleach.

Our findings corroborate the need for relatively long exposure periods and are in agreement with these guidelines.

Time of Freshwater Exposure to Kill Motile T. heterouncinata *Larvae*

Even after transmission of *T. heterouncinata* via transfer of animals and food have been eliminated, transfer of larvae on hands and tools could occur. The large numbers of relatively small production units that typically comprise a California abalone farm necessitates that workers access many different units on any shift, with attendant risk of sabellid transmission. Although the larvae are benthic, larvae could be transferred to hands when sorting or measuring the abalone, and aeration systems within production units result in constant or periodic pulses of air that could dislodge larvae. Culver et al. (1997) recommended rinsing tools and hands with freshwater but did not provide further guidance. Our studies showed that significantly more than a "dip" in freshwater is required to kill all larvae; an exposure period of 32 sec was insufficient. Handwashing periods of longer than 32 sec would be quite cumbersome and the addition of biocides or the use of high-pressure spraying should be considered to provide additional protection.

CONCLUSION

Through the cooperation of industry, academia, and State authorities the sabellid infestation of California abalone appears to be nearly, if not completely eradicated, without spread to native gastropod populations. No survey can give complete assurance and higher risk sites should be revisited after several years, as the threat of infestation within non-haliotid gastropod populations is real. Proper implementation of the freshwater control techniques discussed in this paper will reduce the spread of any cryptic infestations that may still exist at abalone farms or display facilities.

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