Symbionts of the giant red sea cucumber, *Parastichopus californicus* (Stimpson, 1857), with some implications for culture of the host

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Abstract

Commercial fishing impacts on giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) populations in Washington State, USA have prompted the development of hatchery methods for and commercial-scale aquaculture of the species. As these practices grow, it will become increasingly important for culturists to be familiar with naturally occurring *P. californicus* symbionts. For the first time, all of the known symbionts of the giant red sea cucumber are briefly reviewed. Furthermore, new data are presented concerning the ecology of a commensal scaleworm and an endoparasitic snail. This information can be used by culturists and hatchery workers when establishing health management plans for and identifying possible triggers of harmful conditions affecting reared *P. californicus*.

Introduction

The giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) is fully exploited in shallow (depth ≤ 36 m relative to mean lower low water), inland marine waters of Washington State in the United States of America (USA) (Carson et al. 2016; Mueller 2016). In fact, commercial fisheries have resulted in local depletions of *P. californicus* in some management areas of the state, prompting natural resource authorities to develop aquaculture methods and stock enhancement plans for the species (PSI undated; Williams 2014). While scientists have pursued experimental aquaculture of *P. californicus* in Alaska, USA and British Columbia (BC), Canada, mostly in conjunction with other marine species (Ahlgren 1998; Paltzat et al. 2008; Hannah et al. 2013), it has not been until recently that researchers have demonstrated the plausibility of commercial-scale aquaculture of the giant red sea cucumber (DFO 2014; Royal 2016; Suhrbier et al. 2016). As the practice develops beyond experimentation, it will become increasingly important for culturists to be aware of the natural occurrence of *P. californicus* symbionts (Yanong and Erlacher-Reid 2012; Blaylock and Bullard 2014). Endosymbionts are referred to as *infecting* a host, whereas ectosymbionts *infest* a host (Overstreet and Lotz 2016). General terms and quantitative descriptors of symbiont populations (e.g. *prevalence* or *intensity* of), especially those of parasites, follow Bush et al. (1997). Following is the first (albeit brief) comprehensive review of the associations between *P. californicus* and its known symbionts. In addition, novel information is provided concerning the ecology of two rather charismatic symbionts of the giant red sea cucumber. In the end, some implications of these relationships are discussed for the benefit of sea cucumber culturists and hatchery personnel.

Review of giant red sea cucumber symbionts

Bacteria

Until recently, reports of bacterial infections in wild and cultured sea cucumbers have been rare in the scientific literature (Jangoux 1987a; Eeckhaut et al. 2004; Wang et al. 2004; Deng et al. 2009; Liu et al. 2010). There is no documentation of a natural occurrence of bacteriosis in *P. californicus* (DFO 2014); however, the immune response of the giant red sea cucumber has been well documented for nearly 100 years. For example, an early description of *P. californicus* coelomocyte form and function was provided by Kindred (1924). Boolootian and Giese (1958) confirmed Kindred’s (1924) findings and added to the number and type of coelomocytes involved in the *P. californicus* immune response. Conversely, Hetzel (1963) reexamined the coelomic structures reported by the others, refined their descriptions of number, form and function, and hypothesized (correctly) that

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holothurian “brown bodies” were not another type of coelomocyte; rather, the yellowish-brown granules were the product of an immune reaction. Hetzel later went on to describe the formation of brown bodies in *P. californicus* and other sea cucumbers (Hetzel 1965). Dybas and Fankboner (1986) demonstrated the strong immune response of *P. californicus* to a variety of marine bacteria (gut-associated) injected into the coelomic cavity of the sea cucumber, including phagocytosis, the formation and elimination of brown bodies, the degradation of encapsulated material, and the bactericidal activity of the lysosomal enzyme, acid phosphatase. The authors surmised that the antibacterial response in *P. californicus* was a survival mechanism that coincided with the seasonal aestivation process where gut-associated bacteria might be exposed to the sea cucumber. Finally, in studying the coelomucts overlying the rectum of *P. californicus*, Shinn et al. (1990) suggested that, besides helping maintain hydrostatic pressure within the body, the coelomucts provided a pathway for brown bodies to exit the coelom, thereby facilitating excretion and the immune response of the giant red sea cucumber.

**Nonbacterial symbionts**

Like many sea cucumbers, *P. californicus* has a rich assemblage of nonbacterial symbionts — representing several taxonomic groups — associated with it (Jangoux 1987a, b). Table 1 summarizes these nonbacterial symbionts and extends Jangoux’s lists (1987a, 1987b) to include protozoans and a crustacean symbiont of the giant red sea cucumber; their classifications follow those recently reported for eugregarines and coccidians (Kopečná et al. 2006; Wakeman and Leander 2012), ciliates (Lynn and Strüder-Kypke 2002; Gao et al. 2012), flatworms (Van Steenis-Kiste et al. 2013), endoparasitic gastropods (Colgan et al. 2007), scale-bearing polychaetes (Struck et al. 2011; Norlinder et al. 2012; Norlinder 2013), and pea crabs (Ng et al. 2008; Tsang et al. 2014; Palacios-Theil et al. 2016). Some nonbacterial symbionts have been documented in the past few years or decades, whereas others have been recognized for at least a century or more (Table 1).

**Eugregarines and Coccidians**

Protozoan symbionts are common in holothurians (Jangoux 1987a; Eeckhaut et al. 2004); however, information on disease-forming varieties is limited (e.g. Massin et al. 1978). For example, Fankboner and Cameron (1985) described blistering on the viscera of *P. californicus* during its annual aestivation process. The blisters contained masses of sporocysts from unidentified eugregarines; their presence was considered normal for the late visceral atrophy phase. Furthermore, MacCallum et al. (2001) mentioned finding only mature oocysts of an unidentified, apicomplexan coccidian in the respiratory tree and cloaca of a captive specimen of *P. californicus* in British Columbia, Canada. Given that there was no apparent reaction to the oocysts by the host, Bower (2004a) ranked the symbiont as one of negligible regulatory significance to Canadian natural resource authorities, citing that prevention and control of the coccidians would be impractical.

**Ciliates**

On the other hand, with the advent of commercial-scale aquaculture elsewhere, protozoan ciliates have become potentially problematic for cultured sea cucumbers (Wang et al. 2004; Long et al. 2006). The giant red sea cucumber harvests at least two species of these symbionts (Table 1), including one scuticociliate, *Boveria subcylindrica* (Stevens, 1901), that shares the same genus as a disease-causing agent in Asia (Wang et al. 2004; Long et al. 2006). Stevens (1901) remarked that *B. subcylindrica* and a larger sporotrich ciliate, *Licnophora macfarlandi* (Stevens, 1901; but see expanded description by Balamuth 1941), inhabited the respiratory trees of all but one of the 100+ giant red sea cucumber she examined (prevalence ≥ 99%) from the vicinity of Monterey Bay, California, USA. Furthermore, she observed that the smaller *B. subcylindrica* was readily consumed by the larger *L. macfarlandi*, and that there was interannual variation in the intensities of both species. In a natural state, impacts of the ciliates on *P. californicus* are unknown (Jaekle and Strathmann 2013). Stevens (1901) concluded that the two ciliates had no or little impact on their host, which was echoed a century later by MacCallum et al. (2001), who discovered unidentified ciliates in two captive specimens of *P. californicus* from BC, Canada. Since there were no apparent reactions to the unidentified ciliates by the host, Bower (2004b) ranked these symbionts to be of negligible regulatory significance to Canadian natural resource authorities as well, stating that prevention and control of them would be impractical, especially given the ubiquity of the unidentified ciliates.

**Flatworms**

Symbiotic flatworms are widespread in sea cucumbers; *P. californicus* being no exception (Jangoux 1987b). The seminal 1980s work of G. L. Shinn involving the relationships between Northeast Pacific echinoderms and their symbiotic flatworms was reviewed in detail by Jangoux (1987b), so will be only briefly discussed here for the giant red sea cucumber. There are two fully-described umagillid flatworms infecting *P. californicus*: *Anoplodium hymaenae* (Shinn 1983) inhabits only the coelom, whereas *Wahlia pulchella* (Kozloff and Shinn 1987) inhabits the intestine (Table 1). The former is considered to be parasitic (Shinn 1983, 1985a, b), whereas the latter’s endosymbiotic role is not clearly defined (Shinn
Endoparasitic snail

The shell-less, endoparasitic snail, *Enteroxenos parastichopoli* (Tikasingh, 1961) (Fig. 1 B), was likely first observed infecting *P. californicus* more than 120 years ago (Harrington and Griffin 1897), but it would take decades before the eulimid snail was correctly identified as a new symbiont of the holothurian (Tikasingh 1960) (Table 1). Studies of *E. parastichopoli* have primarily focused on histological aspects of the gastropod (Tikasingh 1962) and its systematics (Tikasingh and Pratt 1961; Kincaid 1964). As its name implies, *E. parastichopoli* infects only the giant red sea cucumber (Tikasingh 1961).

Early reports suggest that the symbiont enters the host through the latter’s alimentary tract or anus, eventually making its way out through the gut wall, and finally attaching itself to the anterior end (and coelomic side) of the host’s intestine where the hermaphroditic snail develops and matures (Tikasingh 1960, 1962, 1962; Kincaid 1964). Presumably, infective stages of the parasite are evacuated from the host when *P. californicus* undergoes aestivation or evisceration (Tikasingh 1962; Kincaid 1964). Neither Tikasingh (1962) nor Kincaid (1964) detected larval *E. parastichopoli* in plankton samples; yet Kincaid (1964) anecdotally described the shelled juvenile of *E. parastichopoli*, comparing his observations with a congener of the symbiont. Canadian researchers (Cameron 1985; Cameron and Fankboner 1989) provided the foundation for understanding host-commensal relationships among polychaetes (Martin and Britayev 1998). Additional studies focused on the taxonomy (morphological and molecular), anatomy, reproduction and successful culture of *A. pulchra* (Pettibone 1953; Pernet 1998, 1999, 2000). And similar to *E. parastichopoli*, Canadian researchers (Cameron 1985; Cameron and Fankboner 1989)

Scaleworm

Perhaps the most widely recognized symbiont of *P. californicus* is the scaleworm *Arctonoe pulchra* (Johnson, 1897) (Fig. 1 A). The two have been inexorably paired since the scaleworm was first identified in the 1890s (Johnson 1897; Pettibone 1953; Kozloff 1996); this is especially true for infestations in the San Juan Islands, Washington State, USA (Pernet 1999) (hereafter, San Juan Islands). *A. pulchra* is commensal also with congeners of *P. californicus* (Dimock and Davenport 1971; Lambert 1986). Early studies of *A. pulchra* focused on its behavior in the presence of the giant red sea cucumber, including the scaleworm’s ability to recognize its host (Davenport 1950; Davenport and Hickok 1951; Dimock and Davenport 1971). In fact, Davenport and the others’ research on *Arctonoe* spp. provided the foundation for understanding host-commensal relationships among polychaetes (Martin and Britayev 1998). Additional studies focused on the taxonomy (morphological and molecular), anatomy, reproduction and successful culture of *A. pulchra* (Pettibone 1953; Pernet 1998, 1999, 2000). And similar to *E. parastichopoli*, Canadian researchers (Cameron 1985; Cameron and Fankboner 1989)
Table 1. Nonbacterial symbionts of the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) classified to lowest taxonomic rank possible based on their original description in the scientific literature. Taxonomic ranks above genus and species were based on recent molecular phylogenies for those groups of organisms listed (see text for references).

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus and species</th>
<th>Type</th>
<th>Host site</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td>Apicomplexa</td>
<td>Conoidasida</td>
<td>Eugregarinorida</td>
<td>NA</td>
<td>NA</td>
<td>Unidentified eugregarine</td>
<td>Viscera</td>
<td>Fankboner and Cameron (1985)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Apicomplexa</td>
<td>Conoidasida</td>
<td>Eucoccidiorida</td>
<td>NA</td>
<td>NA</td>
<td>Unidentified coccidian</td>
<td>Respiratory tree; cloaca</td>
<td>MacCallum et al. (2001)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Ciliophora</td>
<td>Spirotrichea</td>
<td>Licnophorida</td>
<td>Licnophorida</td>
<td>Licnophora macfarlandi</td>
<td>Spirotrich ciliate</td>
<td>Respiratory tree</td>
<td>Stevens (1901); Balamuth (1941)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Ciliophora</td>
<td>Oligo-hymenophorea</td>
<td>Thigmotrichida</td>
<td>Boveriida</td>
<td>Boveria subcylindrica</td>
<td>Scuticociliate</td>
<td>Respiratory tree</td>
<td>Stevens (1901)</td>
</tr>
<tr>
<td>Protozoa</td>
<td>Ciliophora</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Unidentified ciliates</td>
<td>Respiratory tree</td>
<td>MacCallum et al. (2001)</td>
</tr>
<tr>
<td>Animalia</td>
<td>Platyhelminthes</td>
<td>Rhabditophora</td>
<td>Rhabdocoela</td>
<td>Umagillida</td>
<td>Anoplodium hymanae</td>
<td>Flatworm</td>
<td>Coelom</td>
<td>Shinn (1983)</td>
</tr>
<tr>
<td>Animalia</td>
<td>Platyhelminthes</td>
<td>Rhabditophora</td>
<td>Rhabdocoela</td>
<td>Umagillida</td>
<td>Wangia pulchella</td>
<td>Flatworm</td>
<td>Intestine</td>
<td>Kozloff and Shinn (1987)</td>
</tr>
<tr>
<td>Animalia</td>
<td>Annelida</td>
<td>Errantia</td>
<td>Phylodocida</td>
<td>Polynoidae</td>
<td>Arctonoe pulchra</td>
<td>Scaleworm</td>
<td>Exterior body wall (i.e. skin)</td>
<td>Johnson (1897)</td>
</tr>
<tr>
<td>Animalia</td>
<td>Mollusca</td>
<td>Gastropoda</td>
<td>Sorbeconcha</td>
<td>Eulimida</td>
<td>Enteraxenos parasichopoli</td>
<td>Endoparasitic snail</td>
<td>Intestine</td>
<td>Harrington and Griffin (1897); Tikasingh (1961)</td>
</tr>
<tr>
<td>Animalia</td>
<td>Arthropoda</td>
<td>Malacostraca</td>
<td>Decapoda</td>
<td>Pinnothereida</td>
<td>Opisthopus transversus</td>
<td>Pea crab</td>
<td>Cloaca</td>
<td>Rathbun (1894); Rathbun (1898)</td>
</tr>
</tbody>
</table>

NA = not available.
were the first to attempt an assessment of how widespread *A. pulchra* infestations might be in juvenile *P. californicus*. Lastly, fecal pellet analyses revealed that, while infesting *P. californicus*, *A. pulchra* fed mostly on crustaceans and polychaetes (Pernet 1998). Approximately one-third of scaleworms examined by Pernet (1998), however, contained unidentified tissue; he found no indication of the symbiont feeding on its host (e.g. giant red sea cucumber ossicles were not observed in fecal pellets).

**Pea crab**

At the close of the 19th century, a small crustacean was discovered infesting a large gastropod in southern California, USA, which is the southern extent of the range of *P. californicus* (Lambert 1986). The pinnotherid crab *Opisthopus transversus*, was formally described and named by Rathbun (1894), who went on to provide additional details on the sexual dimorphism of the pea crab and a host list comprising three additional species, including *P. californicus* (Rathbun 1918). Decades later, the relationship between *Parastichopus* and *O. transversus* was confirmed by others (Ricketts and Calvin 1968; Schmitt et al. 1973), the pea crab’s range was expanded (Hopkins and Scanland 1964), and the list of host species nearly quadrupled in number (Beondé 1968). From the literature, it can be inferred that: 1) *O. transversus* is the only pinnotherid crab infesting *P. californicus* (Schmitt et al. 1973); 2) once a symbiotic relationship is established, *O. transversus* displays a strong affinity for its host (Ricketts and Calvin 1968; Schmitt et al. 1973), the pea crab’s range was expanded (Hopkins and Scanland 1964); 3) more than one host may be required to complete the life history of *O. transversus*; and 5) different hosts may satisfy different nutrient requirements for the pea crab to develop and reproduce successfully (Beondé 1968; Campos 2016). Alternatively, *O. transversus* may just be experiencing a “Goldilocks moment” in time where, evolutionarily, the species is new to symbiosis and is still determining which host is “just right” (sensu Beondé 1968).

**Symbionts and sea cucumber culture: Gleaning new insights by extending the work of others**

Surveys of the prevalence of *P. californicus* symbionts in the wild are few. Besides Shinn’s (1985) work with the flatworm *A. hymanae*, Cameron (1985) (and later, Cameron and Fankboner 1989) provided useful information on the prevalence of *A. pulchra* and *E. parastichopoli* associated with juvenile and subadult giant red sea cucumber (≥ 4 years old) from BC, Canada; however, the latter’s results were based on small sample sizes from a limited number of localities. Recently, Mueller (2016) had the opportunity to collect and inspect a greater number of *P. californicus* of varying sizes and ages, including mature individuals (≥ 5 years old), compared with Canadian researchers. Because his study plan included dissecting every sea cucumber collected for sex determination and maturity, Mueller (2016) was able to check for the prevalence of symbionts across a wide variety of host ages and sizes. The remainder of this paper presents data collected ancillary to a study of the fishery biology of the giant red sea cucumber from the San Juan Islands (Mueller 2016). It is intended to extend the symbiont works of others (e.g. Cameron 1985; Cameron and Fankboner 1989) and to fill in some gaps in our understanding of the ecology of *A. pulchra* and *E. parastichopoli*. A discussion follows on how this information may be of use to those participating in the nascent aquaculture of *P. californicus*.

**Materials and methods**

Mueller’s study (2016) took place in the San Juan Islands where *P. californicus* is abundant relative to other marine areas of Washington State (Bradbury et al. 1998; Carson et al. 2016). The holothurian was collected at several points within four regions (west to east): Upright Channel, Rosario Strait, Bellingham Channel, and the junction of Bellingham, Samish, and Padilla bays (hereafter, “Bays” or Vendovi Island) (Table 2).

Using the same voluntary minimum size limit adopted by many commercial harvest divers as a guideline to retain *P. californicus* (i.e. no shorter than ~5 cm on either side of a gloved hand or ~20 cm whole, contracted length), 50 giant red sea cucumber were collected by divers twice monthly from 7 May 2014 to 15 October 2014, and then again from 17 March 2015 to 28 May 2015. Individual *P. californicus* were placed inside ziplock plastic bags and stored on ice inside a large cooler until being processed the following day at a wet laboratory located on the Northwest Indian College (NWIC) campus in Bellingham. At the NWIC wet laboratory, counts of *A. pulchra* and *E. parastichopoli* were recorded opportunistically for each sea cucumber while other biological data were being gathered (Mueller 2016). The symbionts are relatively large compared with others of the giant red sea cucumber, and are easily identified by an observer without the aid of magnification (Fig. 1 A and B); hence, these qualities simplify determining their presence or absence in the field or laboratory. There was no need to distinguish *A. pulchra* from other species of *Arctonoe* because it is the only scaleworm associated with *P. californicus* in this region of the host’s range (Pettibone 1953; Pernet 1999). Furthermore, no indication of size or life stage was recorded for the scaleworm and endoparasitic snail.

By the end of the study, divers had collected 850 *P. californicus* throughout the central-east San Juan
Islands at average depths ranging from 5.2 m to 33.5 m (mean lower low water) (Table 2). Frequency distributions of *A. pulchra* and *E. parastichopoli* were calculated for host collection date and locality, host sex, and age and size of the giant red sea cucumber. Symbiont prevalence was determined after Bush et al. (1997) as the number of hosts infected or infested with one or more individuals of a symbiont species divided by the number of hosts examined for that species. Proportional prevalence of the symbionts were then plotted at various levels (0, 1, and ≥2 organisms per sea cucumber) moving from west to east in the San Juan Islands (Fig. 3A). In addition, the proportional prevalence of *A. pulchra* increased with the age and size of *P. californicus* collected (Fig. 4A); however, the sex of *P. californicus* did not appear to greatly influence the proportional prevalence of the scaleworm. Indeed, 179 of 364 female *P. californicus* were infested by at least one *A. pulchra* (proportional prevalence = 0.49), whereas 150 of 369 male *P. californicus* were infested by at least one scaleworm (proportional prevalence = 0.41). Lastly, at least one *A. pulchra* infested 60 of 117 giant red sea cucumbers of unknown sex (proportional prevalence = 0.51).

The shell-less, endoparasitic snail *E. parastichopoli*, on the other hand, infected up to 30% of *P. californicus* collected in 2014 and 2015. The intensity of *E. parastichopoli* infections were variable; usually, there were no more than one or two endoparasitic snails per sea cucumber, but intensities as high as 42 per sea cucumber were observed (Fig. 1B). Both juvenile and adult stages of the symbiont comprised the highest intensities of infection, whereas lower intensities were characterized by the presence of adult stages only. Like *A. pulchra*, the proportional prevalence of *E. parastichopoli* appeared to be influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, the proportional presence of *E. parastichopoli* increased slightly during spring, but decreased mid-summer 2014, and decreased further by the last sampling date in fall 2014 (Fig. 2B). In terms of host collection locality (Table 2), the highest proportional

Table 2. Localities where the giant red sea cucumber *Parastichopus californicus* (Stimpson, 1857) was collected by divers in the San Juan Islands, Washington State, USA during 2014 and 2015. Depth range (m) of the collection refers to that as per diver depth gauge (uncorrected for tidal elevation), whereas average depth (m) of the collection refers to that relative to mean lower low water (MLLW).

<table>
<thead>
<tr>
<th>Region</th>
<th>Collection point</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Depth range (m, uncorrected)</th>
<th>Average depth (m, MLLW)</th>
<th>No. sea cucumber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upright Channel</td>
<td>Canoe Island</td>
<td>48°33.561'</td>
<td>122°55.438'</td>
<td>6.1 – 15.2</td>
<td>10.7</td>
<td>50</td>
</tr>
<tr>
<td>Upright Channel</td>
<td>Canoe Island</td>
<td>48°33.675'</td>
<td>122°55.433'</td>
<td>5.5 – 12.2</td>
<td>7.0</td>
<td>50</td>
</tr>
<tr>
<td>Upright Channel</td>
<td>Lopez Island</td>
<td>48°33.899'</td>
<td>122°53.529'</td>
<td>6.1 – 19.2</td>
<td>11.3</td>
<td>50</td>
</tr>
<tr>
<td>Upright Channel</td>
<td>Lopez Island</td>
<td>48°33.913'</td>
<td>122°53.526'</td>
<td>6.1 – 24.4</td>
<td>12.8</td>
<td>50</td>
</tr>
<tr>
<td>Rosario Strait</td>
<td>Orcas Island</td>
<td>48°36.087'</td>
<td>122°48.032'</td>
<td>4.6 – 21.3</td>
<td>11.6</td>
<td>100</td>
</tr>
<tr>
<td>Rosario Strait</td>
<td>Blakely Island</td>
<td>48°35.028'</td>
<td>122°47.930'</td>
<td>12.2 – 20.4</td>
<td>14.0</td>
<td>50</td>
</tr>
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<td>Rosario Strait</td>
<td>Cypress Island</td>
<td>48°35.867'</td>
<td>122°43.885'</td>
<td>33.5 – 36.6</td>
<td>33.5</td>
<td>50</td>
</tr>
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<td>Rosario Strait</td>
<td>Cypress Island</td>
<td>48°36.184'</td>
<td>122°43.542'</td>
<td>4.9 – 12.2</td>
<td>9.8</td>
<td>50</td>
</tr>
<tr>
<td>Bellingham Channel</td>
<td>Cone Islands</td>
<td>48°35.564'</td>
<td>122°41.021'</td>
<td>6.1 – 12.2</td>
<td>9.1</td>
<td>77</td>
</tr>
<tr>
<td>Bellingham Channel</td>
<td>Cone Islands</td>
<td>48°35.520'</td>
<td>122°40.579'</td>
<td>3.4 – 15.2</td>
<td>8.5</td>
<td>100</td>
</tr>
<tr>
<td>Bellingham Channel</td>
<td>Sinclair Island</td>
<td>48°36.642'</td>
<td>122°40.768'</td>
<td>5.5 – 13.7</td>
<td>5.2</td>
<td>50</td>
</tr>
<tr>
<td>Bellingham Channel</td>
<td>Sinclair Island</td>
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<td>122°39.983'</td>
<td>6.1 – 12.2</td>
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<td>“Bays”</td>
<td>Vendovi Island</td>
<td>48°36.821'</td>
<td>122°36.886'</td>
<td>6.1 – 21.3</td>
<td>10.4</td>
<td>150</td>
</tr>
</tbody>
</table>

Results

The commensal scaleworm *A. pulchra* infested up to 70% of *P. californicus* collected in 2014 and 2015. The intensity of an infestation was no more than three scaleworms per sea cucumber. Proportional prevalence of *A. pulchra* was ostensibly influenced by *P. californicus* collection date and locality, and age and size of the host (Figs. 2–4). For example, proportional prevalence of *A. pulchra* increased during the spring of both study years, peaking in mid-summer 2014 and again in mid-fall 2014 (Fig. 2A). Between host collection localities, the proportional prevalence of *A. pulchra* varied by as much as 0.2. While no clear longitudinal trend was observed in sea cucumbers with one scaleworm attached, there was a slight increase in the proportional prevalence of *A. pulchra* at higher densities (i.e. ≥2 polychaetes per sea cucumber) moving from west to east in the San Juan Islands (Fig. 3A).
Figure 2. Proportional prevalence of symbionts of the giant red sea cucumber *Parastichopus californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection date. Fifty giant red sea cucumbers were collected on each date. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

Figure 3. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host collection locality (ordered west to east). The number of giant red sea cucumbers collected by locality is indicated parenthetically. A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.

Figure 4. Proportional prevalence of symbionts of the giant red sea cucumber *P. californicus* in the San Juan Islands, Washington State, USA at three levels of intensity (0, 1, and ≥ 2 organisms per sea cucumber) by host age (years) and size (cm, indicated parenthetically). The numbers of giant red sea cucumber collected for each age and size group were as follows: age 2, \( n = 16 \); age 3, \( n = 107 \); age 4, \( n = 158 \); age 5, \( n = 161 \); and age 6+, \( n = 408 \). A: Proportional prevalence of the scaleworm, *A. pulchra*. B: Proportional prevalence of the endoparasitic snail, *E. parastichopoli*.
prevalence of endoparasitic snails occurred at Lopez Island (Upright Channel) and in the vicinity of Sinclair and the Cone islands (Bellingham Channel; Fig. 3B). And while the proportional prevalence of *E. parastichopoli* in immature *P. californicus* (age = 2–4 years) fluctuated between 0.20 and 0.25, the proportional prevalence of endoparasitic snails in mature sea cucumbers (age ≥ 5 years) fell below 0.15 (Fig. 4B). Regarding sex of the host, 50 out of 364 female *P. californicus* were infected with at least one endoparasitic snail (proportional prevalence = 0.14), whereas 56 of 369 male *P. californicus* were infected with at least one *E. parastichopoli* (proportional prevalence = 0.15). In terms of giant red sea cucumbers of unknown sex, 34 out of 117 were infected with at least one endoparasitic snail (proportional prevalence = 0.29).

Ultimately, irrespective of host collection date and locality, and age and size of *P. californicus*, there appeared to be an inverse relationship between the proportional prevalence of the commensal scaleworm and the proportional prevalence of the endoparasitic snail: whenever or wherever there was an increase in the proportional prevalence of *A. pulchra*, there was a subsequent decrease in the proportional prevalence of *E. parastichopoli* and vice versa (Figs. 2–4).

**Discussion**

At present, the symbiotic community of the giant red sea cucumber (Table 1) does not appear to affect its host too profoundly in the wild (Stevens 1901; Tikasingh 1962; Fankboner and Cameron 1985; Pernet 1998; Bower 2004a, b); however, when placed into an aquaculture setting, one or more of the naturally occurring symbionts of *P. californicus* could become pathogenic or become a pathway or vector for disease (Blaylock and Bullard 2014; Shinn et al. 2015; Overstreet and Lotz 2016). For example, Wang et al. (2004) reported high intensities of a protozoan ciliate inhabiting the respiratory tree of cultured *Apostichopus japonicus* (Selenka, 1867) [likely *Boweria labialis* (Ikeda and Ozaki, 1918)] that weakened the host and occasionally led to the ejection of the sea cucumber’s coelomic contents. In fact, Long et al. (2006) reported secondary bacteriosis in *A. japonicus* as a result of *B. labialis* infections. Furthermore, crustaceans (Brachyura, Copepoda, and Isopoda) living in sympathy with cultured *A. japonicus* and *Holothuria scabra* (Jaeger, 1833) were identified as potential sources of high mortality in the sea cucumbers (Wang et al. 2004; Lavitra et al. 2009). Moving forward, culturists of *P. californicus* should be particularly aware of the scuticociliate *B. subcylindrica*, and in the southern extent of the host’s range, the pea crab *O. transversus*. Although the latter was described as leaving its host periodically to forage (Beondé 1968), the dynamics between host and symbiont could change in an intensive culture setting.

Prior to this study, data on the natural prevalence of *A. pulchra* were limited. For example, Cameron (1985) (and later, Cameron and Fankboner 1989) collected and inspected between 11 and 42 juvenile *P. californicus* (age ≤ 1 year; whole, contracted length < 2 cm) from each of 14 samples taken across four localities in BC, Canada. The author(s) did not report the total number of juvenile giant red sea cucumbers collected, nor did they report the number collected by locality. It can be assumed, however, that the minimum total number of juvenile giant red sea cucumbers collected by them was ≥ 154 (i.e. 11 sea cucumbers X 14 samples). Of all these, only 3 of the 42 juvenile giant red sea cucumbers collected during one trip to one locality were infested with a single, small *A. pulchra* (proportional prevalence = 0.071). Extending this exercise to the regional scale (i.e. all four localities combined), the proportional prevalence of *A. pulchra* on juvenile *P. californicus* can be estimated as the number of infested giant red sea cucumbers (*n* = 3) divided by the total number of holothurians from all 14 samples (*n* range = 11–42 *P. californicus* per sample); or, put another way, the estimated regional proportional prevalence of the scaleworm on juvenile giant red sea cucumbers during Cameron’s (1985) study was between 0.005 [= 3/(42 X 14)] and 0.019 [= 3/(11 X 14)]. In contrast, Pernet (1998) noted a 10-fold increase (or more) in the proportional prevalence of *A. pulchra* (= 0.20) infesting adult-size *P. californicus* from the San Juan Islands, yet both of these examples fall short of the summertime peak (proportional prevalence = 0.70) reported in the present study. Such spatiotemporal differences in the prevalence of symbionts have been widely reported throughout the animal kingdom (e.g. Martin and Britayev 1998; Timi and Poulin 2003; Hoffmann et al. 2016).

Data on the natural prevalence of *E. parastichopoli*, while also limited, have at least been better documented than those for the scaleworm. For example, Harrington and Griffin (1897) reported that 3 of the 30 or 40 *P. californicus* that were examined were infected by endoparasitic snails (proportional prevalence = 0.07–0.10), while Tikasingh (1960) found that just 14 out of 276 giant red sea cucumbers examined in mid-summer to early fall were infected by *E. parastichopoli* (proportional prevalence = 0.05). Furthermore, Jangoux (1987b), citing Lützen’s (1979) findings, tabulated intensities of approximately three *E. parastichopoli* per host in 37 out of 244 *P. californicus* examined (proportional prevalence = 0.15), whereas Cameron and Fankboner (1989) reported a proportional prevalence of 0.09 during August 1983 (6 out of 63 subadult *P. californicus* aged 2–4 years) inspected were infected by *E. parastichopoli*, and no
Interspecific interactions among symbionts have been investigated in both laboratory and natural settings (Pugachev 2000; Timi and Poulin 2003; Fenton et al. 2014). Hypotheses regarding other modes of host–symbiont contact between the giant red sea cucumber and the endoparasitic snail were never made. Only in the past decade was an alternative documented: Altnöder et al. (2007) provided photographic evidence of microscopic, shelled larvae of an endoparasitic snail attached to the skin of its holothurian host. Regarding the possible interaction between _A. pulchra_ and _E. parastichopoli_, it is proposed that the scaleworm preys on the shelled larvae of the endoparasitic snail when the former encounters the latter attached to the skin of _P. californicus_. The two symbionts might also encounter each other in the buccal area of the host where _A. pulchra_ and _E. parastichopoli_ each other in the buccal area of the host where _A. pulchra_ meets _E. parastichopoli_ (Pugachev 2000; Timi and Poulin 2003; Fenton et al. 2014). To summarize, this paper provides the essential first step of reviewing known symbionts of the giant red sea cucumber and delves into the ecology of two of them on multiple levels (time, space and host), providing some possible directions for future research. For example, does the higher proportional prevalence of _E. parastichopoli_ infecting _P. californicus_ of unknown sex (0.29) vs those of male and female giant red sea cucumbers (0.15 and 0.14, respectively) indicate a negative impact on reproductive capacity of the host? Current knowledge of the relationship between endoparasitic gastropods and their echinoderm hosts suggests this may be unlikely (Jangoux 1987b); still, it is difficult to imagine that a moderate to severe infection of _E. parastichopoli_ (Fig. 1B) has no ecological consequences for _P. californicus_. And what drives higher the proportional prevalence of _A. pulchra_ in mature _P. californicus_ (≥ 5 years old) compared with younger giant red sea cucumbers? The host's body size? The host's reproductive status?

In conclusion, the diversity of symbionts associated with the giant red sea cucumber will challenge future _P. californicus_ culturists and hatchery workers with predicting the effects of disease or pest outbreaks and subsequent treatment protocols (Fenton et al. 2014; Shinn et al. 2015). While knowing the symbiont composition in _P. californicus_ (Table 1) is an essential step in “biosecurity” (i.e. minimizing the risk of introducing or transmitting disease or pest agents) of the host species (Yanong and Erlacher-Reid 2012), other lessons to be learned include improved understanding of how symbiont
diversity affects infections or infestations, how sea cucumber culture affects genetic diversity of *P. californicus* (and subsequently, its relationships with symbionts), natural patterns in symbiotic communities across different scales, and symbiotic interactions at the host level (Johnson et al. 2015). This information can be used by culturists and hatchery workers when establishing health management plans for, and identifying possible triggers of, harmful conditions affecting reared *P. californicus* (Wang et al. 2004; Blaylock and Bullard 2014; Overstreet and Lotz 2016).

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**References**


