

## Historical and Modern Invasions to Port Phillip Bay, Australia: The Most Invaded Southern Embayment?

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**ABSTRACT:** Port Phillip Bay (PPB) is a large (1900 km<sup>2</sup>), temperate embayment in southern Victoria, Australia. Extensive bay-wide surveys of PPB have occurred between 1803 and 1963. In 1995/96 the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Centre for Research on Introduced Marine Pests (CRIMP) undertook an intensive evaluation of the region with the aims of developing a comprehensive species list of native and introduced fauna and contrasting previous bay-wide assessments with a current field survey in order to detect new incursions and discern alterations to native communities. Two methods were used to meet these aims: a re-evaluation of regional museum collections and published research in PPB to identify and determine the timing of introductions; and field surveys for introduced benthic (infauna, epifauna, and encrusting) organisms conducted by CRIMP between September 1995 and March 1996. The historic component of PPB invasions groups into four periods based on significant shifts in trade activities: exploration/colonization (pre-1839), immigration (1839-1851), Gold Rush (1852-1860), and modern mechanisms (including aquaculture; 1861-present). Invasions within PPB appear to be increasing, possibly due to an increase in modern shipping traffic and an increase in aquaculture (historically associated with incidental introductions); however, the records of extensive biological surveys suggest that this may in part be an artifact of sampling effort. As expected, the majority of introductions are concentrated around the shipping ports of Geelong and Melbourne. Recent incursions into the region include *Undaria pinnatifida*, *Codium fragile* ssp. *tomentosoides*, *Asterias amurensis*, *Schizoporella unicornis*, and *Pyromaia tuberculata*. Port Phillip Bay is presented as one of the most invaded marine ecosystems in the Southern Hemisphere.

Key words: survey, introduction mechanism, vector, invasion rate, invasion history, Australia

### INTRODUCTION

The threat to biodiversity by introduced species has long been recognized for island systems (Elton 1958). As the only island continent, Australia has developed stringent barrier controls to limit the entry of nonindigenous organisms. This holds true for terrestrial and freshwater systems, and more recently for marine systems. Despite these efforts, Australia has experienced numerous invasions on par with the spectacular international invasions of the Atlantic comb jelly, *Mnemiopsis leidyi*, into the Black Sea, the zebra mussel, *Dreissena polymorpha*, into the North American Great Lakes, and the Asian clam, *Potamocorbula amurensis*, into San Francisco Bay, California. In the late 1980s, the northern Pacific seastar, *Asterias amurensis*, was identified in the Derwent Estuary of Tasmania. *A. amurensis* has attained an estimated population of 28 million and

more recently has spread (most likely via coastal shipping) to Port Phillip Bay (PPB) where current population estimates are of similar magnitude (~30 million). Similarly, the edible Japanese kelp, *Undaria pinnatifida*, introduced near Triabunna, Tasmania, in the mid-1980s, has since spread to a region of approximately 100 km along the coast and was discovered in PPB in 1996. Other high-profile invaders include the Mediterranean fanworm, *Sabella spallanzanii*, the Asian alga, *Codium fragile* ssp. *tomentosoides*, and feral settlement of the maricultured Pacific oyster, *Crassostrea gigas*.

In 1995, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Centre for Research on Introduced Marine Pests (CRIMP) began a two-fold effort to determine the extent of invasions in Australian coastal waters. First, the Australian National Introduced Species Port Surveys Program was established in conjunction with the Australian Association of Ports and Marine Authorities (AAPMA), in which 62 international ports of

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first call are being surveyed using a consistent protocol (Hewitt and Martin 1996). Second, the Port Phillip Bay Introduced Species Study was begun to provide a detailed analysis of the invasion history and introduced species status of a major Australian embayment. PPB was selected based on several factors: its long history of use by maritime trade extending back to the early 1800s; extensive surveys and evaluations for the physical and biological characteristics; numerous scientific collaborators in the immediate vicinity; and CSIRO's previous work in the region relating to taxonomy, distribution, and ecology of the biota.

This paper provides a short synopsis of the results of Port Phillip Bay Introduced Species Study (Hewitt *et al.* 1999), which was a collaborative effort involving Victoria, CSIRO, and New Zealand scientists and represents one of the most thorough investigations of the introduced species status of a single embayment in the world, particularly the Southern Hemisphere.

#### METHODS

A review of the vectors for species transfer, historical trade activities, and shipping patterns into PPB was conducted by CRIMP. The bioregion scheme developed by the International Union for the Conservation of Nature (IUCN; Kelleher *et al.* 1995) was used to aid in evaluating species origins and changes in trade route patterns through time. These bioregions are based on marine physical properties (*e.g.*, salinity, temperature, ocean currents) with secondary regard to biological criteria. An additional classification was created for cosmopolitan species (defined as a species whose native distribution was described as being greater than five IUCN bioregions).

Reviews of major groups were commissioned with specific emphasis on those groups for which taxonomic expertise was available in Victoria (Table 1). The taxonomic experts were requested to review the literature and re-evaluate the museum and personal collections with an eye towards introduced and cryptogenic marine and brackish water species. If necessary, additional field collections were encouraged to produce a comprehensive and authoritative review of the introduced and native status of the biota. We made no attempt to direct the experts in the assignment of species to native, introduced, or cryptogenic status. We recommended using as a guide the ten-point criteria of Chapman and Carlton (1991, 1994)

Table 1. Taxonomic experts, target groups, and target habitats for the taxonomic review component of the Port Phillip Bay Introduced Species Study.

Taxonomic expert	Organization	Target Group	Target Habitat
J. Lewis	Defense Science and Technology Organisation (DSTO)	Macroalgae	All
M. Lockett <sup>1</sup> & M. Gomon <sup>2</sup>	<sup>1</sup> University of Technology, Sydney, <sup>2</sup> Museum of Victoria	Fish	All
M. Keough <sup>3</sup> & J. Ross <sup>4</sup>	<sup>3</sup> University of Melbourne, <sup>4</sup> CRIMP	Fouling species	Hard substrates
J. Watson	Marine Science and Ecology	Hydroids	All
S. Boyd	Museum of Victoria	Molluscs	Soft substrates
R. Wilson	Museum of Victoria	Polychaetes	Soft substrates
G. Poore	Museum of Victoria	Crustaceans	Soft substrates
T. O'Hara	Museum of Victoria	Echinoderms	Soft substrates

to aid in the identification of introduced species and Carlton (1996a) as a guide for cryptogenic species.

A field-sampling program was initiated and carried out by CRIMP (1995-1996) to fill any apparent gaps in the geographic or sampling coverage of previous surveys. This field-sampling program was supplemented by an introduced species survey of the Port of Geelong undertaken by the Marine and Freshwater Resources Institute (MAFRI; Currie *et al.* 1997).

Collection methods were consistent with the protocols developed for the Australian National Introduced Species Port Surveys Program (Hewitt and Martin 1996) with the following differences:

- pile scrapings were performed in a qualitative fashion to represent the fouling community across all depths;
- qualitative visual surveys towing divers on a manta board along 100-m transects;
- beach transect cores were collected at depths of 0, 1, 2, 5, and 10 m depth along a transect perpendicular to the beach;
- beam trawl tows were conducted at 1, 2, 5, and 10 m depth parallel to shore for a known duration (5 min) or length (100 m);
- a small version of a CSIRO seamount sled was developed to sample benthic infauna and epifauna at depths of 5, 10, 15, 20, and 25 m depending on site depth;
- no algae were collected or preserved during this survey.

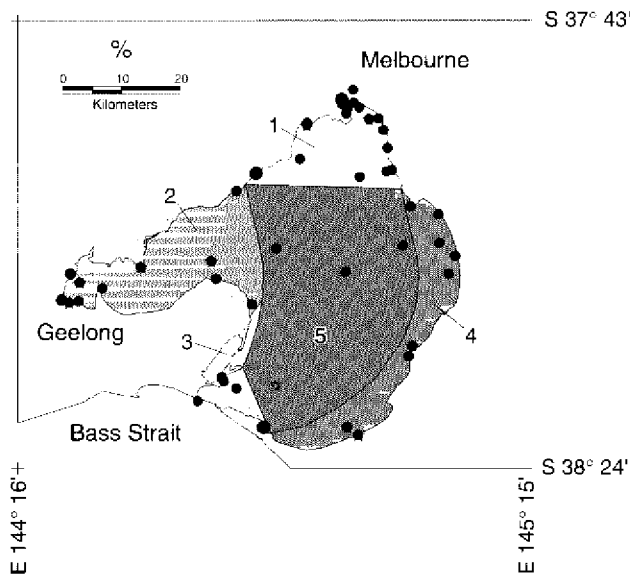


Figure 1. Port Phillip Bay, Australia regions (1–5) and CRIMP sampling locations (for detailed information on locations of specific sampling techniques see Hewitt *et al.* 1999). Note the major shipping ports of Geelong and Melbourne and the connection of sampling effort in these areas.

The geographic extent of PPB (1900 km<sup>2</sup>) precluded a systematic survey of all locations and habitats; instead, a targeted survey was performed in regions of high historic and modern shipping traffic with an overview of the remaining regions (Figure 1). Consequently, the regions were not sampled with equal effort; port regions (1 and 2) were sampled with much greater intensity. The sampling methods used ensured a comprehensive coverage of both hard and soft habitats.

Survey efficacy was evaluated by comparing the target list developed by taxonomic experts from museum and literature reviews with specimen collections from the CRIMP survey. All specimens from the CRIMP survey were identified to the lowest possible taxonomic unit and then verified by the taxonomic consultants that developed the target list, or against a reference specimen provided for that purpose.

## RESULTS

Trading activities in PPB group into four periods (see Campbell and Hewitt 1999a for further details): exploration/colonization (pre-1839), immigration (1839–1851), Gold Rush (1852–1860), and a modern era (1861–present). The history of European influence in Australia is relatively short (Crosby 1986). Despite the long history of aboriginal culture in the region,

the history of biological invasions most likely begins with European contact, however the first biological collections did not begin until the early 1840s and no detailed surveys were conducted until the 1860s. Consequently, while the following presentation of trading patterns broken into four periods provides a context to evaluate invasions, biological surveys did not begin until the mid-1800s and consequently an evaluation of the modern period (1861–present) is presented.

### EXPLORATION/COLONIZATION (PRE-1839)

Sealing and whaling operations were established in the Bass Strait islands by 1796, often using Western Port (the embayment to the immediate east of PPB) as a home base (Shaw 1997). These sealers and whalers were typically from North America, and frequently had contact with Asia (Shaw 1997). British entry into the Port Phillip Heads by John Murray of the *Lady Nelson* in 1802 led to the eventual establishment of a convict colony in 1803 (Shillinglaw 1972). Trade during this period was largely with other Australian colonies; however, periodic visits from Great Britain occurred. From 1803 to 1835, only three ocean-going vessels entered the bay (Shaw 1997). By 1839, regular intra- and inter-colony (South Australia, New South Wales, and Tasmania, Australia; New Zealand) trade routes were established and international routes to Great Britain. Due to the East India Company's monopoly of British trade between the Cape of Good Hope and the Straits of Magellan, there was limited direct trade between British colonies until the British Parliament repealed the British/China trade monopoly laws in 1834 (Staples 1966; Bach 1976). During this period, international vessels trading with PPB followed the Admiralty and later the Great Circle routes. Vessels originating in Europe would typically travel to South America (Rio de Janeiro), South Africa, then to Australia (with some exceptions to trade in India).

### IMMIGRATION (1839–1851)

Free British immigrants arrived in Melbourne in 1839 from Sydney; however, the *David Clarke* arrived from Great Britain later in that year (Strahan 1994). During 1839, 11,500 immigrants arrived at the Point Ormond quarantine station in PPB (Shaw 1997). Pacific trade began in 1840, specifically catering for the demand of Newcastle coal in California (Bach 1976). The repeal of British Navigation laws in 1849

allowed foreign vessels entry into British colonial ports. Simultaneously the signing of the Treaty of Nanking ceded Hong Kong to Great Britain and opened Chinese Ports to British residence and trade (Lubbock 1933).

#### THE GOLD RUSH (1852–1860)

The announcement of gold in Victoria was made in 1851 (shortly after the Port Phillip District separated from Victoria), despite discovery in 1849. This discovery had much the same effect as the California gold strike: the population swelled from <40,000 to 416,000 in five years (Bach 1976; Wild 1950) with immigrants from all continents often abandoning the vessels to rot. Port facilities expanded to meet the needs of a burgeoning population and new domestic (coastal) and international trade routes were opened.

#### MODERN SHIPPING (1861–PRESENT)

Australian shipping tonnage was 93% British until the early 1900s. As trade became increasingly commercial, more ports of call were added to Conference shipping routes (established routes and cargo). By 1870, the trans-Pacific route went from Melbourne to Honolulu, Vancouver, Seattle, Tacoma, Portland, San Francisco, and Los Angeles before returning to Melbourne (Bach 1976). The opening of the Suez Canal in 1869 and subsequent deepening (1875) led to increasing shipping traffic through the Mediterranean. As has been reviewed elsewhere (Carlton 1985, 1996b), shipping changed considerably between the 1860s and present. The shift from wooden hulled to steel hulled vessels reduced the transport of marine borers. Simultaneously, the shift from dry ballast (rock, cobble, sand) to water ballast (in steel ships) halted the transport of near shore meiofauna and adult benthic encrusting and epifauna while developing the transport of holo-, mero- and tycho-planktonic organisms. The increased speeds of vessels and advent of more effective anti-fouling paints is believed to have significantly reduced the transport of encrusting and fouling organisms in numbers if not diversity.

The first biological surveys and collections of PPB began after the 1840s for flora (Harvey 1847, 1855, 1858–1863, 1869; Sonder 1852, 1853, 1880; Wilson 1886, 1889, 1890, 1892, 1894, 1895) and benthic fauna with surveys by Wilson, Agardh, Carpenter, Hickson, Spencer, Sedy, and Pritchard among others (Anon 1890, 1892, 1894, 1895). After 1895,

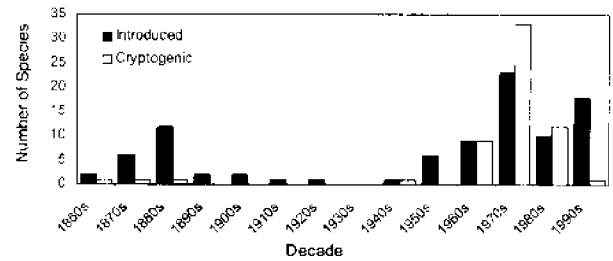


Figure 2. Numbers of introduced and cryptogenic species identified per decade in Port Phillip Bay.

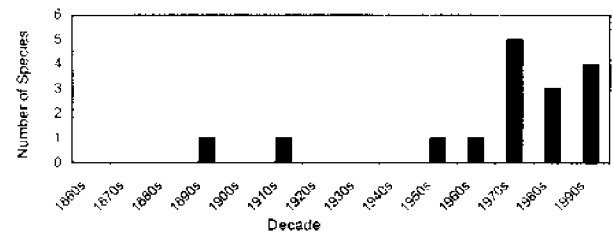


Figure 3. Numbers of identified introduced species from well-known and conspicuous groups (algae, molluscs, and fish) per decade in Port Phillip Bay.

few surveys occurred within the region until the early 1950s. Consequently, despite an increase in the numbers of recognized introduced and cryptogenic species through time (Figure 2) our understanding of the invasion history of PPB is limited by survey intensity. A small subset of well-known and conspicuous groups (*e.g.*, algae, molluscs, fish) that appear to have had a consistent sampling effort provide a mechanism to surmount this difficulty. For these groups a consistent trend towards an increase in introductions post-1950 remains evident (Figure 3).

#### SURVEY RESULTS

The taxonomic experts identified a target list of 182 introduced (92), cryptogenic (65), and possibly introduced (*i.e.*, known from Victoria but not PPB; 25) marine and brackish water taxa. These species spanned nine invertebrate phyla and four algal divisions. Numerous taxa (301) held in the Museum of Victoria collections or collected in previous bay-wide surveys have not been provided specific Latin binomials due to a lack of description or because they were not identifiable. In all cases, these taxa were assigned endemic status by the taxonomic experts rather than cryptogenic. No explicit reasoning was provided for this assignment and their status should be considered questionable without further examination.

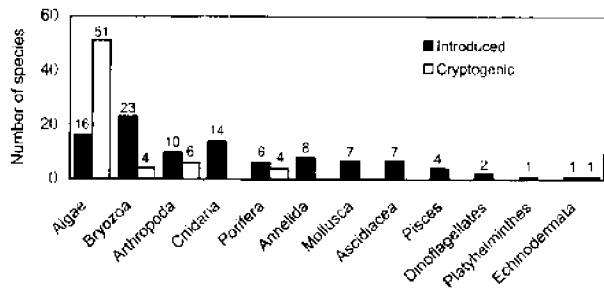


Figure 4. The number of introduced and cryptogenic species in Port Phillip Bay by taxonomic group (does not include unidentified species, see text for explanation).

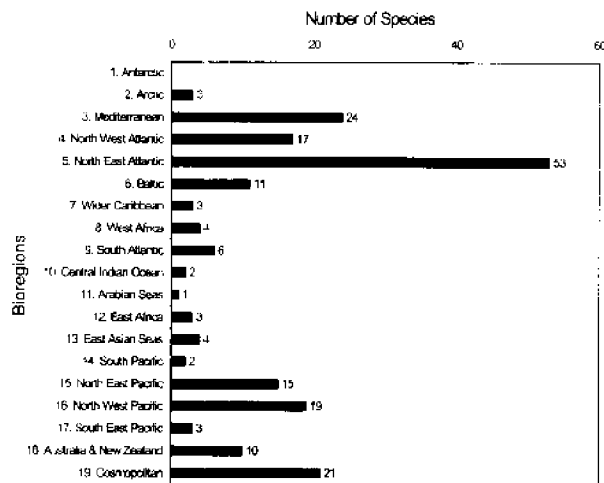


Figure 5. Native bioregions for introduced, cryptogenic and possibly introduced species that have become established in Port Phillip Bay. For more detailed information describing the bioregions see Kelleher *et al.* (1996) and Hewitt *et al.* (1999).

During the course of the CRIMP and the Victorian Marine and Freshwater Resources Institute (MAFRI) surveys, an additional nine introduced taxa were identified, bringing the total introduced and cryptogenic tally to 191 species from 10 phyla and five algal divisions (Figure 4). Forty-nine introduced and cryptogenic species were collected during the CRIMP surveys. Of the 108 animal target species identified by the experts (removing algae), 47.5% of the known target hard-substrate species were detected. Similarly, 48% of the target soft-substrate species were collected by the CRIMP survey.

Using the target species list of 182 taxa developed by the consultants, we evaluated the field surveys conducted by CRIMP and MAFRI. Clearly the extent to which different taxa were readily known and identifiable varied from group to group based in part on the available taxonomic knowledge; however,

all specimen identifications were verified either by the taxonomic expert or by a voucher specimen provided for that purpose. The survey collected differing proportions of target species according to taxonomic group. All fish targets (Chordata – 100%) were collected while other groups such as the Ascidiacea (86%) and Crustacea (80%) were well sampled though not all target taxa were collected. Porifera (66%), Cnidaria (60%), Mollusca (57%), and Bryozoa (52%) were collected with decreasing levels of efficiency, in part suggesting that either the pattern of dispersion in these species was more widespread, or that some of these target taxa identified from historic publications and/or collections may no longer be established in PPB. The Annelida (12.5%) were poorly collected; however, this reflects a lack taxonomic knowledge of the annelids of hard substrate (where the majority of specimens were collected; Wilson 1999) and a reduced emphasis of collection in soft substrate (Campbell and Hewitt 1999b). A single target echinoderm (*Asterias amurensis*) was not collected during the survey and was subsequently found in low numbers (four in two years) prior to achieving outbreak status in 1998 (Talman *et al.* 1999). The majority of detected species (76%) were hard-substrate organisms (bryozoans, crustaceans, cnidarians, and ascidians). Introduced species were detected in all regions of the bay; however, the majority of species were found in port regions (regions 1 and 2; Figure 1).

Species origins can be determined only in retrospect based upon the date of first collection in Port Phillip Bay, the known trade activities prior to collection date, and the known international distribution of the species. Based on these criteria, species introduced to PPB have come from all regions of the world except Antarctica (Figure 5). Historically, the majority originated in the Northeast Atlantic (53) and the Mediterranean (24), while another 21 are cosmopolitan. The North Atlantic (northeast and northwest) appears to have been a consistent and significant donor region for successful invaders. This was the anticipated origin of many species given the long history of trade with Britain and the historic parliamentary limitations on traffic into PPB. A large number of invasions were from the Southern Hemisphere: New Zealand (10), Africa (East Africa, 3; West Africa, 4) and South America (South Atlantic, 6; Southeast Pacific, 3). More recently (post 1950) there has been an increase in the number of introduced

North Pacific species (Northeast Pacific, 15; Northwest Pacific, 19). Since 1990, these North Pacific species represent the single largest group of introductions, many of which have been identified as pest species.

### DISCUSSION

Port Phillip Bay represents one of Australia's largest trade regions historically and in the present. The extent to which this bay has been invaded and the patterns of invasion history provide insight into potential management activities. Invasions within PPB appear to be increasing, possibly due to an increase in modern shipping traffic and an increase in aquaculture activities (historically associated with incidental introductions). As expected, the majority of introductions are concentrated around the shipping ports of Geelong and Melbourne. However, these factors alone cannot account for the increased number of invaders. Trade with new regions, increased vessel traffic, and altered conditions (in Port Phillip Bay) may all have contributed to higher invasion rates (Carlton 1996b). Many of the modern invasions of pest species such as *A. amurensis*, *U. pinnatifida*, and *C. fragile* ssp. *tomentosoides* appear to have been transported as secondary inoculations from other domestic primary inoculation sites.

The numbers of invaders in Port Phillip Bay are high by world standards. Within Australia no other single port or region equals the number of recognized species. In part, this may be due to the effort expended in determining the scale of biological invasions in these locations. A National Port Survey program has been initiated by CRIMP to evaluate the scope of introductions in Australian ports using a consistent and quantitative methodology (Hewitt and Martin 1996). These surveys are designed to provide a snapshot of the current invasion diversity rather than the complete history achieved by the Port Phillip Bay Introduced Species Study due to the extensive coverage by expert taxonomists.

The number of reported introduced and cryptogenic marine and brackish water species in Port Phillip Bay exceeds similar numbers from anywhere in the world. While Cohen and Carlton's study of San Francisco Bay and Delta region (Cohen and Carlton 1995) reports a greater overall introduced and cryptogenic species richness (212 introduced and 123 cryptogenic species), it includes salt marsh and freshwater species. It is difficult to compare surveys

with significantly different methodologies and approaches; however, if the total numbers are restricted to only marine and brackish water species (excluding salt marsh species), San Francisco Bay has approximately 138 introduced species (Cohen *et al.* 1998 present 95 species). This may reflect a stronger trading history of the region or may indicate a higher regional susceptibility to invasions. Regardless, the findings presented here suggest that Port Phillip Bay is one of the most invaded marine ecosystems in the Southern Hemisphere.

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## Factors Limiting the Spread of the Introduced Mediterranean Mussel *Mytilus galloprovincialis* on Washington's Outer Coast

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Key words: mussel, *Mytilus galloprovincialis*; eastern Pacific, invasion, ecological resistance, survivorship, range expansion

### INTRODUCTION

In the wake of fish stock overexploitation around the world (Naylor *et al.* 1998), aquaculture is increasingly being developed as a potentially more sustainable source of protein. Global aquaculture production has more than doubled in weight and volume in the last decade, and now accounts for more than one quarter of all seafood consumed by people (Naylor *et al.* 1998). One consequence of this growth in aquaculture is an increase in the transfer and spread of culture species around the world. In order to predict the ecological consequences of these introductions, we need to know where, in a given region, these introduced species will spread.

One of the most commonly used bivalves in raft culture is the Mediterranean blue mussel *Mytilus galloprovincialis*. Globally, *M. galloprovincialis* is native to the Mediterranean Sea and has spread through accidental transport and intentional introduction to Asia, North America, and the southern hemisphere (Carlton in press). In South Africa, the spread of *M. galloprovincialis* has led to a dramatic reorganization of the intertidal zone. It has replaced the native mussel *Aulacomya ater*, causing a change in algal diversity and limpet population structure, and has been correlated with an increase in oystercatcher fecundity (Griffiths *et al.* 1992). In California, *M. galloprovincialis* has replaced the native sibling species, *M. trossulus*, at a number of intertidal locations (Geller 1999).

Most recently, *M. galloprovincialis* has been introduced both intentionally and accidentally to the Pacific Northwest. It is cultured at several shellfish farms in Puget Sound, Washington, where it is favored over the native sibling species, *M. trossulus*,

which succumbs to a hemolytic neoplasia before reaching market size. In addition, *M. galloprovincialis* has been identified in ballast water arriving in Oregon from Japan (Carlton and Geller 1993), and is presumably also delivered to ports of Washington and British Columbia. From these numerous sources, *M. galloprovincialis* is spreading in rocky intertidal communities of the Pacific Northwest (Suchanek *et al.* 1997). Its invasion here will be limited in part by its ability to survive wave action, desiccation stress, and predation. Here, I experimentally compare the ability of the introduced and native *Mytilus* sibling species to survive wave action, desiccation (as tidal height), and predation.

### METHODS

I compared the survivorship of transplanted *M. galloprovincialis* and *M. trossulus* in the rocky intertidal of Tatoosh Island, just off the outer coast of Washington State (48°24'N, 124°41'W). At this site the native California mussel, *M. californianus*, a larger and thicker shelled species, forms conspicuous mid-intertidal beds with ephemeral gaps created by wave and storm disturbance (Paine and Levin 1981). Since *M. californianus* is not a member of the sibling species complex that includes *M. galloprovincialis* and *M. trossulus*, and plays a different ecological role (Suchanek 1978), I did not manipulate it in the current comparison of sibling species. *M. trossulus* is found in two distinct intertidal zones on Tatoosh: in the ephemeral gaps within the *M. californianus* bed, and in a more permanent band above the *M. californianus* (Suchanek 1978, as *M. edulis*).

I transplanted mussels to six sites encompassing a range of wave exposures from high to low. At each site, I planted mussels at two tidal heights: in gaps in the *M. californianus* bed, and in the *M. trossulus* zone

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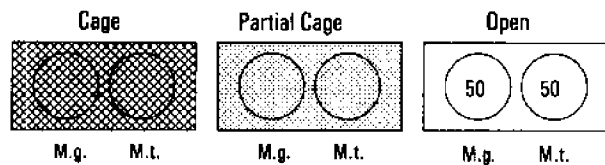


Figure 1. Mussel transplant experimental design, Tatoosh Island, Washington. Fifty individuals of the introduced *Mytilus galloprovincialis* (M.g.) and of the native *M. trossulus* (M.t.) were transplanted in three paired plots. The three plots were haphazardly assigned to cage (i.e., predator exclusion), partial cage (i.e., cage control), and open treatments with no cage. This design was repeated at two intertidal heights (high and mid) at each of six sites over a range of wave exposures (high, medium, low).

above the *M. californianus*. At each height, I planted three patches of 50 mussels of each species, *M. galloprovincialis* and *M. trossulus* (Figure 1). The patches were paired by species, and pairs were haphazardly assigned to one of three treatments: fully caged to exclude predators (primarily the drilling snails, *Nucella emarginata* and *N. canaliculata*); a partial cage to allow predators in and control for unintended caging effects; and an open treatment with no cage (Figure 1). Each patch was covered with fiberglass mesh to allow the mussels to attach byssal threads to the rock. After two weeks, I removed the mesh and monitored mussel survivorship over the next 16 wk. At 2-wk intervals, I counted the number of live mussels, empty shells, and shells with drill holes in each patch. I also surveyed the density of *Nucella* spp. at each pair of transplants. Results are presented for the first six weeks.

### RESULTS AND DISCUSSION

Proportion data were arcsin (square root) transformed and count data were  $\ln(x+1)$  transformed to improve normality. All analyses were conducted using JMP version 3.0 statistical software. Mussel survivorship at six weeks was analyzed by four-factor ANOVA testing for effects of species, tidal height, wave action, and treatment. The proportion of mussels that was washed away was compared for the two species using a Student's *t*-test. The proportion of empty mussel shells with drill holes was regressed linearly against the density of *Nucella* spp. in each patch.

There was no significant difference in survivorship between the two species. Both species survived significantly better in caged treatments, where they were protected from predators, than in open treatments where they were exposed to predation (Table 1; Figure 2A). Both species survived significantly

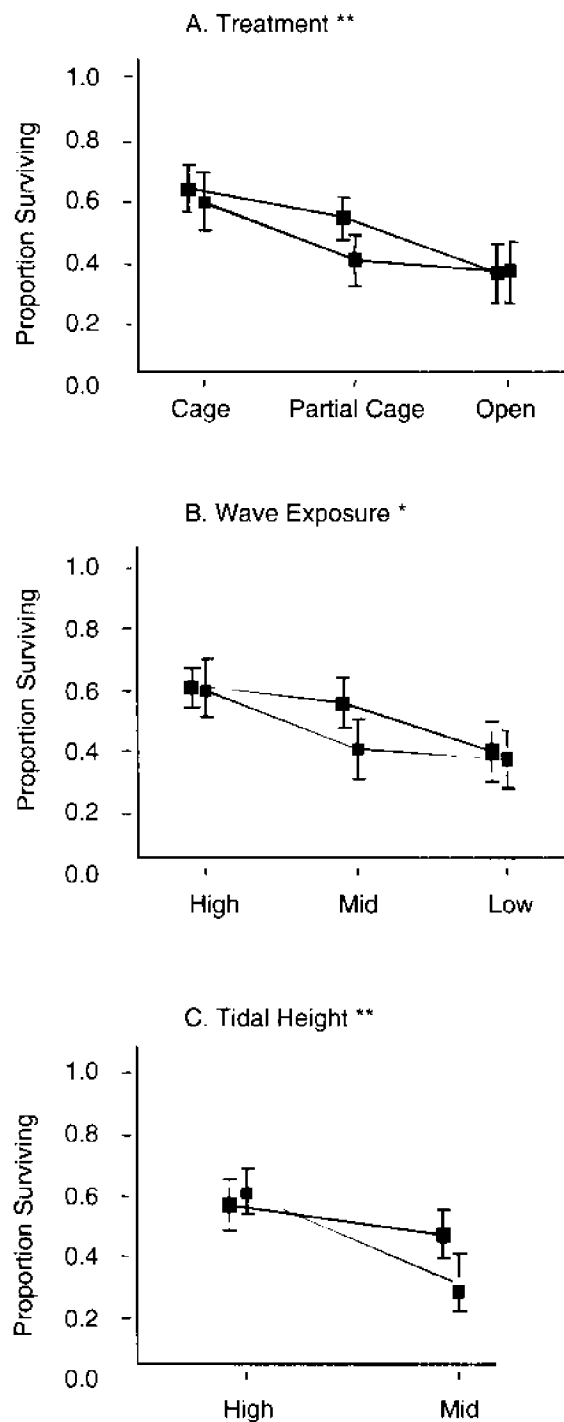


Figure 2. Transplanted *M. galloprovincialis* survived equally with *M. trossulus* in all locations and all treatments on Tatoosh Island, Washington, after six weeks. Both mussels survived significantly better (a) in the cage treatment than in the open treatment; (b) at high than low wave exposure; and (c) at high than low tidal height. Points represent the mean of  $n = 12$  cages (a and b) and  $n = 18$  cages (c)  $\pm 1$  standard error. Significant differences indicated by \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ); horizontal lines indicate means that were not significantly different in post-hoc pairwise comparisons.

Table 1. Proportion of transplanted mussels surviving after six weeks on the intertidal rocky shore of Tatoosh Island, Washington: ANOVA results. There was no significant difference between species (introduced *M. galloprovincialis* vs. native *M. trossulus*). Significant main effects were found for wave exposure (high, medium, low), tidal height (high, mid), and treatment (predator exclusion cage, partial cage, open). See also Figure 2.

Source of Variation	DF	SS	F	p
Species	1	0.092	0.765	0.385
Wave Exposure	2	0.939	3.882	0.026
Tidal Height	1	.232	10.194	0.002
Treatment	2	.300	5.375	0.007

better at high than at low wave exposure, and at high than at low tidal height (Table 1; Figure 2B, C).

Inspection of the empty shells suggested that the primary causes of mortality for these two species may not be the same, for two reasons. First, more of the empty shells of *M. trossulus* ( $43.7\% \pm 4.80$ ) than *M. galloprovincialis* ( $31.8\% \pm 5.02$ ) tended to remain attached to the rock after six weeks (mean  $\pm 1$  S.E.,  $t = 1.871$ ,  $p = 0.066$ ). This suggests that *M. galloprovincialis* may have weaker byssal thread attachments than *M. trossulus*. Second, some of the empty shells had drill holes, indicating that those mussels had been eaten by the predatory snails *Nucella* spp. *Nucella* spp. density varied among sites: at sites with higher *Nucella* spp. density, the proportion of drilled *M. trossulus* shells tended to be higher (slope = 0.30,  $R^2 = 0.11$ ,  $p = 0.084$ ), but the proportion of drilled *M. galloprovincialis* shells did not (slope = -0.25,  $R^2 = 0.05$ ,  $p = 0.339$ ). This trend suggests that the native predatory snail may prefer the native mussel to the introduced mussel. These hypotheses, concerning byssal strength and predator preference, will be further tested in laboratory experiments.

Since *M. galloprovincialis* appears to survive as well as *M. trossulus* over a range of wave exposures and tidal heights, the two species are likely to overlap as *M. galloprovincialis* spreads through the Pacific Northwest. Competitive interactions between the two species may therefore be important in limiting the spread of *M. galloprovincialis*. The present results suggest that local variations in wave exposure and

predator density have the potential to affect the outcome of this competitive relationship. In future field experiments I will compare the growth rates of these two species over a range of conditions. Additional factors that I have not yet addressed include the abiotic variables of water temperature and salinity; and the biotic factors of susceptibility to parasites and disease.

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## The 1998 Puget Sound Expedition: A Shallow-Water Rapid Assessment Survey for Nonindigenous Species, with Comparisons to San Francisco Bay

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**ABSTRACT:** A rapid assessment survey for nonindigenous species at 23 primary stations and eight secondary stations was conducted September 8–16, 1998, in the inland marine waters of Washington State from Blaine, at the Canadian border, to Olympia, in south Puget Sound. The 1998 Expedition team was composed of scientists with both broad and specific taxonomic and regional expertise from universities and local and state agencies. It included core researchers from the four San Francisco Bay Expeditions of 1993–1997, where the survey techniques were developed. Using a variety of sampling methods on marina docks and adjacent shallow water benthic habitats, the 1998 team collected and identified more than 400 native species and 39 nonindigenous species (3 plants and 36 invertebrates)—taxonomic work on the samples is still underway. The number of nonindigenous species collected per site showed no obvious correlation with salinity, temperature or region. We believe that Puget Sound presently hosts more than 50 nonindigenous marine species. This is substantially fewer than the approximately 160 nonindigenous species known to be in the marine and estuarine portions of San Francisco Bay, excluding its associated Delta region. The vast difference in invasion level is noteworthy considering the close shipping links between these two estuaries for the past 150 years, although it is also partly a sampling artifact reflecting the different state of knowledge of nonindigenous species in the two estuaries.

**Key words:** rapid assessment, Puget Sound, Washington, Northwest Straits, San Francisco Bay, Pacific, invertebrate, alga, *Zostera*, *Spartina*, ascidian, crustacea, introduced species, nonindigenous species, estuary

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## INTRODUCTION

Understanding the level of invasion and disturbance in the various ecosystems within which we operate is becoming an increasing priority as general awareness of the disruptive effects of nonindigenous species in terrestrial, freshwater, and marine ecosystems grows. San Francisco Bay is one of the best-studied marine ecosystems in terms of non-native species introduced by human activities over recent centuries (Carlton 1979; Cohen and Carlton 1995; Cohen 1996; Cohen and Carlton 1998). In addition to these well-known studies on San Francisco Bay, a series of four surveys (known affectionately as the San Francisco Bay Expeditions) was completed by a team of biologists during the 1990s. These were week-long rapid assessment surveys for introduced species in the float-fouling communities of the Bay. Following the model of the San Francisco Bay Expeditions of 1993, 1994, 1996, and 1997, we carried out a similar Puget Sound Expedition in the late summer of 1998 to inventory nonindigenous marine species in the float-fouling communities of greater Puget Sound, using the core researchers and methods pioneered in San Francisco Bay between 1993 and 1997.

The rapid assessment survey methods reported here provide estimates of species diversity. The use of rigorous quantitative methods in most modern marine ecological research precludes sampling large areas over a short time for presence or absence of species. Rapid assessment methods have been refined here for the detection of maximum numbers of marine species, allowing flexibility in sampling. Biological invasions are detectable only by looking for species, and become apparent only when accompanied by accurate taxonomic identification of the invading organisms. This rapid assessment method tests whether or not field surveys by taxonomic specialists can detect biological invasions that have been overlooked, unreported, or misidentified in standard, modern ecological studies.

Puget Sound is the southern portion of one of the three largest North American estuary systems. It is a complicated, glacier-produced inlet approximately 150 km long, with many branches providing approximately 3,350 km of shoreline. The Sound has numerous freshwater inflows and a complicated circulation pattern driven by tidal forcing. To its north, Puget Sound merges with the Strait of Juan de Fuca

(which runs west to the Pacific Ocean) and connects with the Strait of Georgia (which separates Vancouver Island from the southern British Columbia mainland) via a patch of American waters sometimes called the Salish Sea or Washington Sound and recently dubbed the Northwest Straits in conjunction with a new American federal marine conservation management initiative. We sampled from south Puget Sound to the northern edge of Washington State in the Salish Sea.

Metropolitan areas are now concentrated on the eastern side of this region. The Puget Sound basin presently supports a population of nearly 4 million people. Southwestern British Columbia includes an additional 3 million. Population growth rates, primarily from immigration, are presently high on both sides of the international border, and are predicted to remain high in the foreseeable future. There is abundant cross-Sound ferry transportation, both public and private, and heavy recreational boat use. The robust present-day shipping trade derives primarily from ports up and down the west coast of North America and from other countries on the Pacific Rim. Major Puget Sound shipping destinations include the deep-water port facilities at Seattle, Tacoma, and Olympia and oil refineries in the Northwest Straits region near Anacortes and Bellingham. Each year, approximately 6000 ships enter the Strait of Juan de Fuca from the Pacific Ocean (Washington State Office of Marine Safety, now Department of Ecology 1996). About half of these enter American ports and the other half turn north into the Strait of Georgia towards Canadian port facilities around Vancouver (Niimi, this volume).

Puget Sound and San Francisco Bay have been tightly connected by shipping since the earliest days of European and Asian settlement of the Puget Sound region in the early to mid-1800s. Some of the earliest Puget Sound-Pacific Rim shipping contacts were made by the Hudson Bay Company in the 1830s, when agricultural and other natural resource products from the Puget Sound region were shipped to Hawaii, China, Australia, and Europe. By the 1850s, logs and lumber from Puget Sound were being shipped in great quantities to San Francisco. Today, both the Puget Sound and San Francisco Bay estuary systems receive enormous amounts of shipping traffic (and associated ballast water) from all over the world, but principally from other Pacific coast North American ports and Pacific Rim countries.

Comparison of the state of marine bioinvasion of San Francisco Bay and Puget Sound is therefore an important and interesting project.

#### MATERIALS AND METHODS

The First Puget Sound Expedition took place September 8–16, 1998. The Expedition team was composed of 15–20 marine scientists with broad and specific taxonomic and regional expertise from several universities and local agencies (Cohen *et al.* 1998). Using a rapid assessment approach, we sampled 23 marinas composed of floating docks (primary sites) and eight intertidal sites (secondary sites) between Olympia and Blaine (Table 1), a distance of about 230 km. The sampling occurred over six days; we identified most of the species collected in the field or immediately afterward at the University of Washington's Friday Harbor Laboratories.

Marinas with floating docks provide important and common substrates for marine organisms in the inside waters of Washington State and British Columbia. Private and public year-round mooring facilities are located abundantly along the shoreline, sometimes accommodating many hundreds of small boats, and frequently offering covered as well as uncovered moorage space. Different organisms can be found under the covered and uncovered portions of these marinas and we sampled both whenever the situation allowed.

Our primary goal in selecting sampling sites was to achieve the widest and most uniform coverage of Puget Sound possible in the four full sampling days available to us; some additional sampling was done from Friday Harbor. The duration of the expedition was determined by time available to the majority of scientists involved. The time was then split approximately in half to allow both field and lab time. With these goals in mind, preliminary visits were made to about 60 potential sites from the Canadian border to the south Sound. Site selection was ultimately based on geographic distribution, with as many different inlets included as possible, and with sites characterized by heavy commercial use as well as by light to heavy recreational use. A few sites were eliminated on the days of sampling for lack of time, because our original schedule was too ambitious. Five sites per day seemed comfortable and realistic, although on three days we managed to sample six sites either by working late, or by sampling the sixth site more superficially. Waning daylight ultimately determined

Table 1. List of stations sampled during the Puget Sound Expedition, September 8–16, 1998.

Station #	Station description	City	County
<b>Primary sites</b>			
1	Port of Everett Marina	Everett	Snohomish
2	Port of Edmonds Marina	Edmonds	Snohomish
3	City of Des Moines Marina	Des Moines	King
4	Harbor Island Marina, near mouth of Duwamish River	Seattle	King
5	Elliott Bay Marina	Seattle	King
6	Fishermen's Terminal	Seattle	King
7	Ole & Charlie's Marina	Tacoma	Pierce
8	Steilacoom Marina	Steilacoom	Pierce
9	Boston Harbor Marina	Olympia	Thurston
10	Port of Shelton Marina	Shelton	Mason
11	Fairharbor Marina	Grapeview	Mason
12	Kitsap Marina	Port Orchard	Kitsap
13	Brownsville Marina	Brownsville	Kitsap
14	Seabeck Marina, Hood Canal	Seabeck	Kitsap
15	Port Ludlow Marina	Port Ludlow	Jefferson
16	Port Hadlock Bay Marina	Port Hadlock	Jefferson
17	Boat Haven Marina	Port Townsend	Jefferson
18	Deception Pass Marina, Whidbey Island	north of Oak Harbor	Island
19	Blaine Marina	Blaine	Whatcom
20	Squalicum Harbor, Port of Bellingham	Bellingham	Whatcom
21	Samish Bay, small float near mouth of Samish River	Edison	Skagit
22	Cap Sante Boat Haven	Anacortes	Skagit
23	University of Washington, Friday Harbor Labs floating dock	Friday Harbor	San Juan
<b>Secondary sites</b>			
1	beach at Steilacoom Marina	Steilacoom	Pierce
2	beach at Boston Harbor Marina	Olympia	Thurston
3	beach at Seabeck, Hood Canal	Seabeck	Kitsap
4	beach north of the Blaine Marina	Blaine	Whatcom
5	lagoon at Port Ludlow Resort	Port Ludlow	Jefferson
6	beach at Port Ludlow Resort	Port Ludlow	Jefferson
7	Padilla Bay – <i>Vaucheria</i> flats east of the Swinomish Channel	east of Anacortes	Skagit
8	Argyle Lagoon	south of Friday Harbor	San Juan

the end of each sampling day. In addition to the 50–60 minutes spent sampling each site, another half hour was required to get the gear put away and samples properly labeled and stored before moving on to the next location.

At each primary site, we sampled a variety of habitats. Float- and dock-fouling organisms were sampled by a variety of simple manual techniques. Tools included hand scrapers, a 1.3-m long-handled scraper

with attached 3-mm steel mesh net, a 2.4-m pole with attached 1-mm nylon mesh net, and a broad array of pans, buckets and other containers. Organisms were collected individually from the substrata, or by scraping and subsequent washing through coarse sieves followed by 1.0-mm or 0.5-mm mesh sieves for final washing. We collected from concrete, styrofoam, and wooden float surfaces as well as ropes, tires, conduit, bumpers, chains, and boat bottoms within reach. At two sites we were able to examine newly removed floats because reconstruction was taking place during our visit, and at another site a heavily fouled rowboat was pulled out for us. A sample of live bay mussels (*Mytilus* sp.) was obtained from each site where they were present; these were later frozen for genetic analysis. Benthic (bottom) and plankton samples were also taken at most dock sites. (Two of the scientists were unable to participate during the first two days of the sampling, but returned to the missed sites and collected plankton and mussel samples within two weeks.) An Ekman grab was used to obtain non-quantitative bottom samples that were sieve-washed (using 1.0- or 0.5-mm meshes as deemed appropriate for the organisms being collected) and sorted on site; unsorted bottom samples were retained for later examination for foraminifera and other microfauna. A custom-made cylindrical benthic sampler fitted with 1-mm stainless steel mesh walls was thrown out on a line and dragged back along the bottom, working like a small benthic sled to collect larger infauna. Vertical plankton hauls were taken with a 0.5-m diameter, 102- $\mu$ m mesh net with a 211- $\mu$ m mesh cod end. Horizontal plankton tows were taken by pulling a plankton net fitted with 125- $\mu$ m mesh alongside each dock. The tows were made close to the float-fouling community in an effort to obtain demersal organisms such as harpacticoid copepods. At least two team members took field notes for the group at each site. Pilings were sampled separately at several sites, and nearby intertidal sites were opportunistically sampled when the tide and shoreline geography allowed. In order to compare similar environments, docks were classified as primary sites, and other sites such as beaches were classified as secondary sites.

Surface temperature and salinity were measured at each dock site. We attempted to use two electronic (Yellow Springs Instruments) meters to obtain depth profiles of temperature and salinity, but these devices produced varying and unreliable readings (as was the experience of the San Francisco Bay Expeditions).

The measurements we preferred were near-surface measurements obtained with two thermometers and two refractometers that agreed to within 0.5°C and 0.5 psu.

At each dock site, we filled a 1-L jar with a representative voucher collection, plus additional samples of material of interest. If laboratory time was scheduled soon after the field work, the samples were kept on ice; otherwise they were preserved on-site in formalin or alcohol for later analysis. Laboratory work was conducted at the King County Environmental Laboratory and the University of Washington's Friday Harbor Laboratories. The voucher collections were all re-examined in the laboratory. Most organisms were identified by team members. Some specimens were retained by individual team members for further study; some were sent to other taxonomic specialists. The voucher collections are currently held by the Washington State Department of Natural Resources, and will ultimately be deposited in an appropriate curated museum collection.

This project was conceived in March 1998, carried out in September 1998 and the results were presented at the First National Conference on Marine Bioinvasions in January 1999, all within 10 months. We had little funding by conventional standards, but the rapid timeline allowed a high level of spontaneity and enthusiasm among the participants. This was integral to the success of the 1998 Puget Sound Expedition. Not all of the data analysis was completed during the Expedition and further identifications are anticipated later at the convenience of the participants.

## RESULTS

We collected and identified more than 450 species of plants and invertebrates, providing a snapshot of the fouling fauna of Puget Sound in late summer, 1998. Most of the species were native (in the sense that they are known or assumed to be present on the Pacific coast of America naturally, that is, prior to the arrival of European colonists). Thirty-nine nonindigenous species were identified (Table 2). Eleven of these nonindigenous species are new records for Puget Sound and several more confirm unpublished reports of established nonindigenous species.

An additional 31 cryptogenic species (those whose native range cannot be determined, thus of *unknown origin*) have so far been identified in samples

Table 2. Nonindigenous species collected by the 1998 Puget Sound Expedition including geographic origins, first records and possible mechanisms of introduction; \*\* indicates a new record for Puget Sound. This list of species is provisional pending further taxonomic work and review by expedition members and associates and includes additions or corrections to a similar Table 2 in Cohen et al. (1998), which it supercedes (see text for sources). First records consisting of written accounts that do not state the date of planting, collection or observation are preceded by the symbol "<". Mechanisms are listed as: OA – with shipments of Atlantic oysters, OJ – with shipments of Japanese oysters, SF – in ship fouling or boring, SB – in solid ballast, BW – in shipballast water or seawater system, MR – planted for marsh restoration or erosion control (mechanisms given in parentheses are considered less likely).

Taxon	Native Range	First Record <sup>1,2</sup> Pacific Coast	First Record <sup>1,2</sup> Puget Sound	Possible Mechanism of Introduction
<b>Phaeophyceae</b>				
<i>Sargassum muticum</i> (Yendo, 1907) Fensholt, 1955	Japan	1944	1948 <sup>3</sup>	OJ
<b>Anthophyta</b>				
<i>Spartina anglica</i> C.E. Hubbard, 1968	England	1961-1962	1961-62	MR
<i>Zostera japonica</i> Ascherson and Graebner, 1907	W Pacific	1957	1974 <sup>4</sup>	OJ
<b>Foraminifera</b>				
<i>Trochammina hadai</i> Uchio 1962	Japan	1971	1971 <sup>5</sup>	BW, SF, OJ
<b>Cnidaria: Hydrozoa</b>				
<i>Cordylophora caspia</i> (Pallas, 1771) (= <i>Cordylophora lacustris</i> )	Black Sea and Caspian Sea	ca. 1920	ca. 1920	BW, SF
<b>Cnidaria: Anthozoa</b>				
<i>Diadumene lineata</i> (Verrill, 1869) (= <i>Haliplanella luciae</i> )	probably Asia	1906	< 1939	OA, SF
<b>Annelida: Polychaeta</b>				
<i>Hobsonia florida</i> (Hartman, 1951)	NW Atlantic	1940 <sup>6</sup>	1940 <sup>6</sup>	?
<i>Pseudopolydora paucibranchiata</i> (Okuda, 1937)	Japan	1950	1993 <sup>7</sup>	BW, SF
<b>Mollusca: Gastropoda</b>				
<i>Batillaria attramentaria</i> (G. B. Sowerby II, 1855) (= <i>B. zonalis</i> , = <i>B. cumingi</i> )	Japan	1924	1924	OJ
<i>Crepidula fornicata</i> Linnaeus, 1758	NW Atlantic	1905	1905	OA
<i>Myosotella myosotis</i> (Draparnaud, 1801) (= <i>Ovatella myosotis</i> )	Europe?	1871	1927	OA (SB, SF)
<b>Mollusca: Bivalvia</b>				
<i>Crassostrea gigas</i> (Thunberg, 1793)	Japan	1875	1875	OJ
<i>Mya arenaria</i> Linnaeus, 1758	NW Atlantic	1874	1888-1889	OApr 7560
<i>Nuttallia obscurata</i> (Reeve, 1857)	Japan, Korea (China?)	1991 <sup>8</sup>	1993 <sup>8</sup>	BW
<i>Venerupis philippinarum</i> (Adams and Reeve, 1850) (= <i>Ruditapes philippinarum</i> = <i>Tapes japonica</i> )	NW Pacific	1924	1924	OJ
<b>Arthropoda: Crustacea: Copepoda</b>				
**Chionostomatid copepod	?	?	1998	
<b>Arthropoda: Crustacea: Cumacea</b>				
<i>Nippoleucon hirumensis</i> (Gamo, 1967)	Japan	1979	mid-1990s <sup>9</sup>	BW
<b>Arthropoda: Crustacea: Isopoda</b>				
<i>Limnoria tripunctata</i> (Menzies, 1951)	?	1871 or 1875	1962 <sup>10</sup>	SF

Anthropoda: Crustacea: Amphipoda					
<i>Amphithoe valida</i> (Smith, 1873)	NW Atlantic	1941	1966 <sup>11</sup>		BW, OA, SF
** <i>Caprella mutica</i> (Schurin, 1935) (= <i>C. acanthogaster</i> )	Sea of Japan	1973-1977	1998		BW, OJ
<i>Corophium acherusicum</i> (Costa, 1857)	N Atlantic	1905	1974-1975		OA, SF
<i>Corophium insidiosum</i> (Crawford, 1937)	N Atlantic	1915	1949 <sup>2</sup>		OA, SF
<i>Eochelidium</i> sp.	Japan or Korea?	1993?	1997		BW
<i>Grandiferella japonica</i> (Stephensen, 1938)	Japan	1966	1977 <sup>13</sup>		BW, OJ, SF
<i>Jassa marmorata</i> (Holmes, 1903)	NW Atlantic	1941	1990 <sup>7,14</sup>		BW, SF
** <i>Melita nitida</i> (Smith, 1873)	NW Atlantic	1938	1998		BW, OA, SB, SF
** <i>Parapleustes derzhavini</i> (Gurjanova, 1938)	W Pacific	1904	1998		SF
Kamptozoa / Entoprocta					
** <i>Barentsia benedeni</i> (Foettinger, 1887)	Europe	1929	1998		OJ, SF
Ectoprocta / Bryozoa					
<i>Bowerbankia</i> sp. "gracilis"	NW Atlantic?	<1923	<1953		OA, SF
<i>Bugula</i> sp. 1	?	?	1993		?
** <i>Bugula</i> sp. 2	?	?	1998		?
** <i>Bugula stolonifera</i> (Ryland, 1960)	NW Atlantic	<1978	1998		SF
** <i>Cryptosula pallasiana</i> (Moll, 1803)	N Atlantic	1943-1944	1998		OA, SF
<i>Schizoporella unicornis</i> (Johnston, 1847)	NW Pacific	1927	1927		OJ, SF
Urochordata: Tunicata					
<i>Botrylloides violaceus</i> (Oka, 1927)	Japan	1973	1977		OJ, SF
<i>Botryllus schlosseri</i> (Pallas, 1766)	NE Atlantic	1944-1947	1970s <sup>15</sup>		OA, SF
** <i>Ciona savignyi</i> (Herdman, 1882)	Japan	1985	1998		BW, SF
** <i>Molgula manhattensis</i> (DeKay, 1843)	NW Atlantic	1949	1998		BW, OA, SF
** <i>Styela clava</i> (Herdman, 1881)	China to Sea of Okhotsk	1932-1933	1998		BW, OJ, SF

<sup>1</sup>most pre-1979 dates are from Carlton (1979).<sup>2</sup>most post-1979 dates from Cohen and Carlton (1995).<sup>3</sup>first Puget Sound record from the San Juan Islands (Stagle 1956).<sup>4</sup>first Puget Sound record from Padilla Bay, Washington (Phillips and Shaw 1976; Harrison and Bigley 1982).<sup>5</sup>first Pacific Coast and Puget Sound record by McGinn et al. (2000).<sup>6</sup>first Pacific Coast and Puget Sound record from Everett, Washington (Banse 1979).<sup>7</sup>first Puget Sound record from Eagle Harbor in 1993 by the Washington State Department of Ecology (K. Welch, pers. comm).<sup>8</sup>first Pacific Coast record at White Rock, B. C. (Forsyth 1993). first Puget Sound record from San Juan Islands, Washington (A. Scruton, pers. comm.).<sup>9</sup>first Puget Sound record from Seattle, Washington, mid-1990s by Jeff Cordell (unpublished).<sup>10</sup>first Puget Sound record in Quayle (1965).<sup>11</sup>first Puget Sound record from Hood Canal by Bousfield (in Bousfield and Jarrett 1981).<sup>12</sup>first Puget Sound record from Totten Inlet and Whidbey Island by Shoemaker (1949).<sup>13</sup>first Puget Sound record from Seattle, Washington, 1977 by Fredric H. Nichols (C. P. Staude, pers. comm.).<sup>14</sup>if this species proves to be synonymous with *I. stauderi*, the first Puget Sound record is from Friday Harbor, Washington by Craig Staude (C. P. Staude, pers. comm.).<sup>15</sup>first Puget Sound record from San Juan Islands, Washington (Lambert and Lambert 1998).



from the 1998 Expedition; about half are organisms requiring further work to identify to the species level. When species identification is completed, some of these cryptogenic species may move to the non-indigenous species list. The (ongoing) list of cryptogenic species is available on the web at <http://faculty.washington.edu/cemills/PSrecords.html>.

Salinity at the sites varied between 0 and 34 psu, with all but two of the stations having salinities over 20 psu. Sea water temperatures varied from 12° to 20°C. The number of nonindigenous species collected and identified per site, not counting a number of still-unidentified annelids or peracarids, ranged from zero to eight. Plots of the number of nonindigenous species per site against salinity, temperature, or by region revealed no discernible patterns (Cohen *et al.* 1998).

### DISCUSSION

This rapid assessment survey focused primarily on non-quantitative or semi-quantitative sampling of dock fouling communities (organisms growing on the sides and undersides of floating docks and associated floats, bumpers, tires, ropes, etc.). Field identification of specimens was followed by examination in the laboratory by taxonomic experts on the team. Sampling dock-fouling communities has the following advantages:

- The habitat is easily sampled at low cost and with simple equipment.
- It can be sampled regardless of the tide level.
- There is easy and quick access to a large selection of suitable sites throughout Puget Sound.
- Most sites provide an adequate working area for a sizable team of experts to sample simultaneously, while remaining in verbal contact.
- In many coastal regions, the dock-fouling fauna includes a significant nonindigenous or cryptogenic component.
- Dock-fouling communities are normally extremely diverse and profuse, allowing rapid collection of many species.

The following disadvantages should also be ascribed to dock sampling:

- The communities are mostly restricted to hard and biogenic substrates.
- Many species of nonindigenous macrophytes (seaweeds and vascular plants) are more likely to become established in shallow benthic habitats than on docks and pilings and are therefore missed.

- It is very difficult to sample quantitatively.

We chose to sample in September mostly because that is when the participants were available. Late summer sampling turned out to be perhaps the best possible time, as annuals such as hydroids, bryozoans, and tunicates were well developed. Many of these would not have been present in such abundance at other times of the year. Tunicates were producing large numbers of larvae in September, and the morphology of the larva proved necessary for positive identification of at least one species (*Botrylloides violaceus*). Peracarid crustaceans are also at peak abundance and reproductive maturity in late summer and early fall, providing excellent specimens on which to make specific identification. Many of the hydroids were also reproductive, enabling their identification to species, although a somewhat different, and probably more diverse, set of hydroids would have been found earlier in the year (May and June). The algae would also have been more diverse if we had sampled in May and June; many early species had already died back when we sampled.

The expedition team was remarkable for the amount of local marine invertebrate and plant knowledge and specific taxonomic expertise that was represented. Recognition of newly established alien organisms requires a thorough knowledge of the local fauna and flora to allow recognition of species that are not native to the system. Identification of non-indigenous species requires familiarity with, and access to, the worldwide literature. Less experienced laboratory workers might conveniently assign names already in the Puget Sound literature to similar, new nonindigenous species. Because of their expertise, the ability of the expedition members to recognize non-indigenous species was high. There were, however, several prominent taxa of organisms that we could not identify with the specificity needed to recognize nonindigenous species. Such taxa included some hydroids, which are abundant in fouling communities throughout Puget Sound, but have never been thoroughly worked up locally to the species level; thus, we lacked the background information necessary to draw comparisons during a week-long field trip. Flatworms and sponges could not be positively identified even to genus during the expedition. The low worldwide taxonomic resolution of some of the polychaetes and peracarid crustaceans prevent distinctions between some native and nonindigenous species. Green algae *Ulva* and *Enteromorpha* were

ubiquitous at the waterline at most float sites, but their identification to species is problematic, requiring genetic or ultrastructural taxonomic expertise not available to us.

We are now compiling a master list of nonindigenous species that are presently established in Puget Sound. We anticipate that it will include a few more than 50 species. This list will be substantially shorter than the 78 species currently listed for all Washington State and adjacent marine waters by the Washington State Department of Fish and Wildlife (<http://www.wa.gov/wdfw/fish/nuisance/ans4.htm>). Previous lists of nonindigenous species in Puget Sound include that of Carlton (1979), who acknowledged 29 nonindigenous marine species in the region covered by the Puget Sound Expedition; Elston (1997), whose list of 31 nonindigenous species includes shared inland waters of Washington and British Columbia; and Ruiz and Hines (1997), who listed 67 nonindigenous species in the marine and estuarine waters of Washington State and British Columbia. Our expedition is the first extensive field-based study since Carlton's 1976 and 1977 fieldwork in Puget Sound and the Strait of Georgia. We collected 24 nonindigenous species not on Carlton's list, 30 species not on Elston's list, and 14 species not on the Ruiz and Hines list or the Washington State Department of Fish and Wildlife website (which is based largely on the Ruiz and Hines list).

The San Francisco Bay and estuary system now supports more than 230 nonindigenous species (Cohen and Carlton 1998). Of these, about 160 are in marine and estuarine habitats comparable to what is here considered as greater Puget Sound. The Puget Sound area has received considerably less attention than San Francisco Bay; the list of approximately 50 nonindigenous species in Puget Sound does not include fish or salt marsh organisms, as are listed for San Francisco Bay. Some of the difference between the lengths of these lists therefore continues to be a reflection of effort by scientists looking for nonindigenous species, but Puget Sound has not yet received many of the devastating new organisms now well established in San Francisco Bay. We plan to extend our studies of Puget Sound to additional habitats (see below). No list of freshwater nonindigenous species has been generated for rivers in Puget Sound that might be comparable to the 90 known nonindigenous species in the Delta region of the San Francisco Estuary (A. Cohen, unpubl. data).

While marinas provided accessible platforms for sampling subtidal communities close to the surface that are not subject to tidal exposure (thus considerably lengthening the daily sampling window), we largely missed sampling the extensive intertidal mudflat/sandflat habitat typical of much of Puget Sound. Brief stops at Samish Bay and Padilla Bay added several nonindigenous species to our list and indicate that these habitats must be sampled in the future to gain a more comprehensive picture of bioinvaders.

The northernmost site sampled in 1998 was determined by the Canadian border, which provides a politically, but not ecologically, meaningful boundary. Puget Sound and the Strait of Georgia are tightly linked ecosystems that share much of the same flora and fauna and will together share the burdens and environmental changes that come with invasions and establishment of new marine organisms from other regions.

Further complementary expeditions are planned for Puget Sound and the Strait of Georgia. Much of the original Puget Sound Expedition team plans to reassemble to sample mudflats and beaches in Puget Sound and Willapa Bay in May 2000. A team of (mostly Canadian) biologists sampled float-fouling communities on both the mainland and Vancouver Island sides of the southern Strait of Georgia in late winter 1999 and plans to repeat this exercise in late summer in the near future (C. Levings, pers. comm.).

At least one-quarter of the nonindigenous species now known to be present in Puget Sound have been previously overlooked, unreported, or misidentified. The possible impacts of these invasions thus remain unexplored by modern ecological research. Our findings demonstrate the value of rapid assessment surveys by trained systematists as a useful technique to detect marine or aquatic bioinvasions. Comparison of nonindigenous species in Puget Sound and San Francisco Bay also highlights the magnitude of the continued threat from interstate ballast water transport up and down the west coast.

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the U.S. Environmental Protection Agency, and Mary Mahaffy of the U.S. Fish and Wildlife Service, and finally to the much-nicer-than-predicted weather during our long days in the field. The Washington State Department of Natural Resources provided vans, mileage, ferry fares, meal and lodging reimbursements, equipment, supplies, and employee time. The U.S. Fish and Wildlife Service, Puget Sound Water Quality Action Team, and King County Department of Natural Resources provided funds, equipment, or employee time that contributed to the success of the Expedition. Availability of space at the University of Washington Friday Harbor Laboratories and the Western Washington University Shannon Point Marine Laboratory was appreciated. Eugene Kozloff, Bruno Pernet, Scottie Henderson, Mary McGann, Doris Sloan, Craig Staude and Wim Vervoort all provided expert assistance with species identification.

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# The Freshwater Expansion and Classification of the Colonial Hydroid *Cordylophora* (Phylum Cnidaria, Class Hydrozoa)

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**ABSTRACT:** *Cordylophora* spp. is a colonial hydroid occurring in brackish and freshwater habitats. Records indicate that the distribution of this hydroid is expanding globally by increased boat travel and ballast discharge. *Cordylophora* spp. occurrences are becoming more common in freshwater habitats probably due to an increase in salts from runoff. This paper provides an updated distribution of *Cordylophora* spp. populations in several freshwater systems in the United States. DNA analyses along with interbreeding experiments using several populations of *Cordylophora* spp. are proposed to address a possible taxonomic discrepancy between two of the five documented species of *Cordylophora*, *C. caspia* and *C. lacustris*. *Cordylophora* spp. poses problems to power plants in Europe and the United States. Methods to curtail hydroid growth in pipes at a local power plant in Morris, Illinois were investigated. Laboratory experiments using cultured colonies indicate that high temperature is most effective (compared to chlorine doses) in killing or curtailing hydroid growth. Hydroids exposed to lower temperatures and chlorine regenerated due to the presence of menonts or resting stages. Elucidation of the overall distribution patterns and genetic structure of various hydroid populations will assist in confirming or modifying the taxonomy of this genus and will provide ecological information for an organism that is becoming a more prevalent invading species.

Key words: hydroid, range, taxonomy, *Cordylophora*

## INTRODUCTION

Evaluation of the distribution, classification, and biofouling potential of the hydroid *Cordylophora* spp. is necessary due to an increased occurrence of this organism in freshwater systems. The purpose of this paper is to provide an updated literature review of the biology and classification of the genus *Cordylophora*. Reasons for the range expansion of the hydroid are addressed and are related to a higher incidence of fouling problems caused by *Cordylophora* spp.

## BIOLOGY OF CORDYLOPHORA

*Cordylophora* spp. (Class Hydrozoa, Family Clavidae) is a colonial, athecate hydroid occurring in fresh water to brackish habitats and therefore is able to live within a wide salinity range from 0 to 20 ppt (Fulton 1962; Green 1968; Roos 1979; Gaulin *et al.* 1986; Pennak 1989; Thorp and Covich 1991) (Figure 1A, 1B). Growth is optimal at 15 ppt salinity with colonies growing up to 5 cm though this varies depending on the habitat (Green 1968; Pennak 1989). Colonies are polymorphic, consisting of polyps specialized for feeding (gastrozooids) or

reproduction (gonophores) and are dioecious with gonophores containing eggs or sperm (Figure 1B). A medusa stage is lacking, while a free-swimming planula larva is released from female gonophores (Pennak 1989). Colonies flourish especially during late spring and summer months with a peak in growth during July, August, and September depending on water temperature (Roos 1979; Journalainen *et al.* 1994; pers. obs.). Colony proliferation via asexual budding can be quite rapid if conditions are favorable. When water temperatures decrease in the fall, the colonies die back and consist simply of stalks, with portions of the coenosarc or structures referred to as menonts inside the perisarc or chitinous covering (Roos 1979) (Figure 1B, 1C). The menonts allow the hydroid to withstand periods of stress and/or cold temperatures. When water temperatures begin to increase in the spring, cells within the menonts begin dividing and new colonies are established. The presence of menonts and the ability for regeneration make this hydroid resistant to environmental changes and persistent in growth when it is responsible for biofouling.

## DISTRIBUTION AND RANGE EXPANSION

*Cordylophora* spp. was first discovered in the Caspian and Black Seas and supposedly came to

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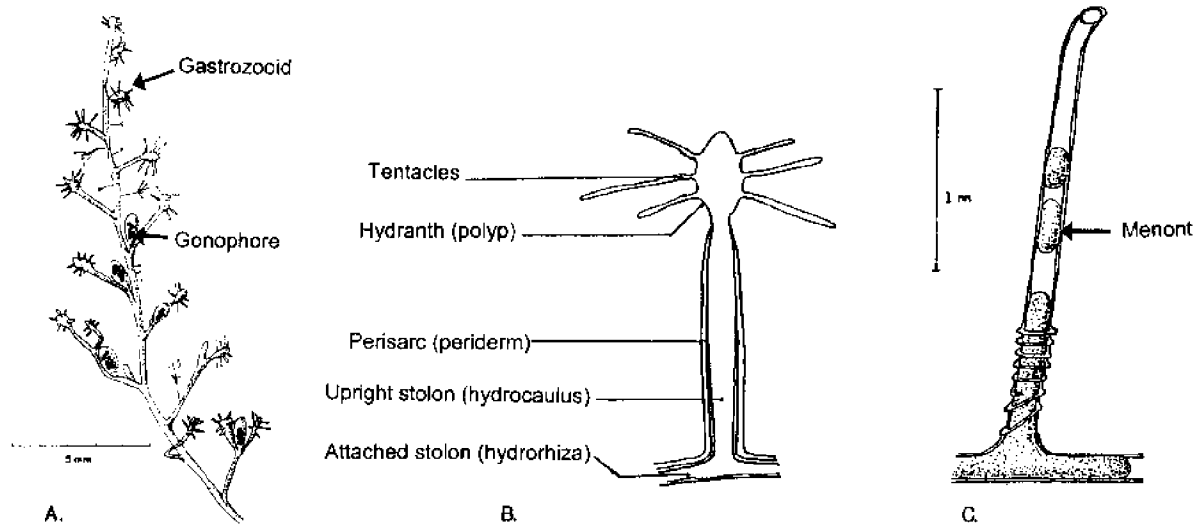


Figure 1. A. Part of a *Cordylophora* colony taken from a *Nuphar* stalk, with gastrozooids, female gonophores and epifauna. Camera lucida drawing (Roos 1979). B. A drawing of the anatomy of an upright stalks and hydrorhiza of *Cordylophora* (re-drawn from Marcum and Diehl 1978). C. Menonts in hydrorhiza and base of hydrocaulus. Camera lucida drawing (Roos 1979).

North America in the late 1800s (Clarke 1878; Thorp and Covich 1991). There are currently five species of *Cordylophora* documented in the literature: *caspia*, *lacustris*, *annulata*, *pusilla*, and *inkermanica* (Roos 1979; Fulton 1962; Calder 1988; Boreo 1987; Marfenin 1983, respectively). Arndt (1984) produced a map of the global distribution of *Cordylophora caspia*. That map has been updated by adding the locations of the other four species along with additional citations for the occurrence of *C. caspia* (Figure 2). *C. annulata* occurs in Bermuda, while *C. pusilla* and *C. inkermanica* occur off the coast of Spain and in the Black Sea, respectively (Marfenin 1983; Boreo 1987; Calder 1988). The remaining locations, and thus the majority of the points on the map, refer either to *C. caspia* or *C. lacustris*. It is apparent from the updated map that the geographical distribution of *Cordylophora* spp. (especially in the United States) is expanding inland in freshwater ecosystems. The global distribution of the hydroid is in part due to its ability to tolerate a wide range of salinity along with increased boat travel and ballast discharge (Roos 1979; Thorp and Covich 1991; Carlton and Hodder 1995). It is also suggested that anthropogenic factors such as pollution or eutrophication are responsible for this expansion by altering salt concentrations in the water (Hubschman 1971). The increased prevalence of this hydroid in freshwater systems is possibly due to an increase in salts (especially chlorides) from runoff (Smith 1989).

#### CLARIFICATION OF CLASSIFICATION

A need for clarification of the taxonomy relating to *C. caspia* and *C. lacustris* is evident by reviewing the literature. Pennak (1989) describes *C. lacustris* as a freshwater hydroid and states that some taxonomists think that *C. lacustris* and *C. caspia* are synonymous. Confusion exists in the literature with some biologists referring to *Cordylophora* as *C. lacustris* while others refer to it as *C. caspia* (Table 1A,B). Some authors equate the two (Roch 1924 as cited by Ramane and Schlieper 1971; McClung *et al.* 1978; Roos 1979; Smith 1989; Cohen *et al.* 1998). Smith (1989) states that *C. caspia* is used more commonly in the European literature. Jormalainen *et al.* (1994) cites

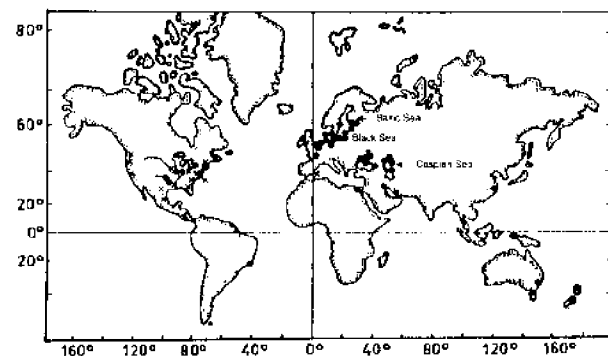


Figure 2. An updated map showing the global distribution of *Cordylophora* spp. (modified from Arndt 1984). The Xs indicate additional locations of the hydroid. There are currently five species of *Cordylophora* documented in the literature: *caspia*, *lacustris*, *annulata*, *pusilla* and *inkermanica*.

Table 1. A summary of the documentation of two species of *Cordylophora* (*C. caspia*=Cc; *C. lacustris*=Cl). Some authors were more specific in providing habitat descriptions and locations. FW= freshwater habitats. Part A lists studies documenting *C. caspia* (Pallas 1771). Part B includes references to *C. lacustris* (Allman 1844).

Author	Part A. Habitat	Location
Arndt 1984	(Cc) Brackish (5-7 ppt)	Iraq
Calder 1971	(Cc) Brackish, S. Chesapeake Bay	Virginia, U.S.
Calder 1976	(Cc) Estuarine	South Carolina, U.S.
Calder 1990	(Cc) Estuarine	South Carolina, U.S.
Calmano et al. 1992	(Cc) Estuarine	Germany
Cohen et al. 1998	(Cc=Cl) Brackish San Francisco Bay	California; Washington, U.S.
Franzen 1996	(Cc) Brackish (5-6 ppt), Baltic Sea	Stockholm, Sweden
Jormalainen et al. 1994	(Cc) Brackish, Aurajoki River	Turku, Finland
Ringelband and Karbe 1996	(Cc) Brackish (10 ppt)	Nord-Ostseekanal, Germany
Roos 1979	(Cc=Cl) Brackish rivers	Amsterdam, Holland
Von Holle and Ruiz 1997	(Cc) Estuarine, Baltimore Bay	Maryland, U.S.
Walton 1996	(Cc) Brackish, Hudson River	New York, U.S.

work by Gaulin et al. (1986) on *C. lacustris* by making reference to *C. caspia*. Green (1968) describes *C. caspia* as growing best at about 15 ppt while its freshwater counterpart has different "proportions" and has been described as *C. lacustris*. Differences in morphology suggest either that *C. caspia* and *C. lacustris* are two different species or that they are synonyms and that a great deal of plasticity in morphology is present. Morphological variation in *C. caspia* is caused by variation in salinity and temperature (Kinne 1956, as cited by Remane and Schlieper 1971). Kelly and Franks (1995) cite work by McClung et al. (1978) indicating variable colony size with increased chloride concentrations. However, the data of McClung et al. (1978) do not concur with Kelly and Franks' results for *C. lacustris* observed in east Texas. Kinne (1956, as cited by Remane and Schlieper 1971) reports *Cordylophora caspia* stalks being taller in brackish water (16.7 ppt) compared to colonies in freshwater. Similarly, Pennak (1989) documents that brackish water colonies of *C. lacustris* reach 20-100 mm in height while freshwater colonies rarely exceed 30 mm. Locally in Illinois, freshwater colonies obtain heights of 10-50 mm (pers. obs., Morris Illinois; Dr. Terry Marsh, pers. comm.). These colonies grow in

Table 1 Continued: References to *Cordylophora lacustris*.

Author	Part B. Habitat	Location
Calder 1968	(Cl) FW/low salinity	Virginia, U.S.
Chain 1980	(Cl) FW, Tamar River (0-10 ppt)	Cotechele Quay, England
Clarke 1878	(Cl) Brackish, Curtisi creek	Baltimore, Maryland, U.S.
Fulton 1962	(Cl) FW	Nye Pond, North Falmouth, MA
Gaulin et al. 1986	(Cl) Estuarine	New Hampshire, U.S.
Hubschman 1971	(Cl) FW, Lake Erie	Pennsylvania, U.S.
Kelly and Franks 1995	(Cl) FW, Livingston Reservoir	Huntsville, Texas, U.S.
Lipsev and Chimney 1978	(Cl) FW, Baldwin Lake	Illinois, U.S.
McClung and Davis 1983	(Cl) FW, Concho River	San Angelo, Texas, U.S.
McClung et al. 1978	(Cl=Cc) FW, 5 rivers	Texas, U.S.
Moore 1951	(Cl) Estuarine, Tamar & Ouse River	Norfolk, England
Pennak 1989	(Cl) FW	New Jersey to Gulf of St. Lawrence, Illinois, Arkansas, Arizona, Kansas, Tennessee, Louisiana, U.S.
Siegfried et al. 1980	(Cl) Estuarine, Sacramento River	California, U.S.
Smith 1989	(Cl=Cc) FW	Massachusetts, U.S.
Streever 1992	(Cl) FW, Little River Spring cave system	Florida, U.S.

areas of extreme flow and are at times exposed to slight increases in salinity due to evaporation caused by heating of power plant effluent water. Jormalainen et al. (1994) observed brackish water colonies of *C. caspia* reaching heights of 5 to > 50 mm. Remane and Schlieper (1971) also state that brackish colonies are at "their best" in the number of gonozooids and the number of tentacles on the hydranths; freshwater colonies have fewer and smaller gonophores and eggs. Roch (1924, as cited by Remane and Schlieper 1971) indicates that colonies of *C. lacustris* require more oxygen at lower salinity.

Yet another anatomical or morphological feature that can lead to confusion in classification between *C. caspia* and *C. lacustris* are rings or annulations on the perisarc of the stalk as seen in diagrams presented in the literature. For example, Pennak (1989) and Smith (1989) present diagrams of *C. lacustris* with annulations (Smith also refers to *C. caspia* and *C. lacustris* as synonymous) while Moore's (1951)

diagram of *C. lacustris* lacks rings. Green (1968), Remane and Schlieper (1971), Marcum and Diehl (1978), and Barnes (1994) present diagrams of *C. caspia* without annulations, while Roos (1979) presents a diagram of a colony (*C. caspia* = *C. lacustris*) without annulations and another drawing documenting menonts or overwintering structures with annulations on the stalk. Calder (1971) presents *C. caspia* with annulations.

All of these features, including variation in colony height, numbers of gonophores and tentacles, and the presence or absence of annulations on the perisarc indicate prominent eco-plasticity in colony morphology. This eco-plasticity may be genetically based and may lead to reclassification within the genus *Cordylophora*. The environmental habitats and locations of *Cordylophora* spp., as summarized from the literature, clearly indicate a great deal of habitat variation (Table 1B). With current taxonomic thought considering *C. lacustris* and *C. caspia* as conspecifics, Calder does encourage molecular analyses among populations for clarification (D. Calder, pers. comm.). It is unknown how much morphological variation is plastic and how much is genetically based.

#### FOULING BY CORDYLOPHORA SPP.

*Cordylophora* spp. causes biofouling by colonizing the inner walls of power plant pipes in Europe and the United States (Lipsev and Chimney 1978; Jenner and Janssen-Mommen 1993; Moreteau and Khalanski 1994; A. Cohen, pers. comm.; Folino and Indelicato, unpublished data; M. Khalanski, pers. comm.).

The Collins Generating Station located in Morris, Illinois, south of Chicago, is owned and operated by Midwest Generation (formerly owned by Commonwealth Edison). *Cordylophora* spp. was clogging the intake tunnel, causing blockage of filters and condenser tube sheets at the plant. Funding was granted to the author and student assistants to develop a treatment protocol for curtailing growth of the hydroids in unwanted areas. Colonies obtained from the Des Plaines River in Joliet, Illinois were cultured and exposed to thermal treatments of 35°C, 36.5°C, 37.7°C, and 40.5°C for varying lengths of time ranging from 1 to 8 hr. Several experimental sets of colonies were also exposed to chlorine levels ranging from 0-5 ppm for 20 min, 105 min, and for three 20-min doses in a 24-hr period. Colonies did not survive

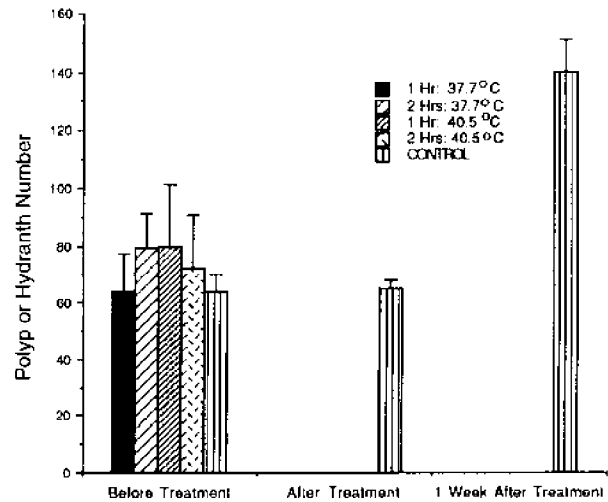


Figure 3. Results of the 37.7 and the 40.5°C treatments. Colonies were exposed for 1 and 2 hrs. Polyp or hydranth number was assessed before, immediately after and 1 week after each treatment. None of the colonies survived.

at the two highest temperatures (Figure 3), while colonies at the lower temperatures and at all of the chlorine concentrations exhibited varying degrees of survival and regeneration relative to exposure time (Folino and Indelicato, unpubl. data). It was clear that the presence of the menonts in the chitinous perisarc allowed for colony regeneration and growth. Thermal treatments appear to be the more effective and ecologically sound approach to addressing this biofouling problem at this particular plant. In other related studies dealing with pests such as *Corbicula fluminea* (asiatic clams) (Cameron *et al.* 1989) or *Drissena polymorpha* (Van Benschoten *et al.* 1995; Harrington *et al.* 1997), chlorine and/or exposure to high temperatures were used to control or curtail growth.

In addition to fouling problems, some have suggested that this hydroid impacts freshwater/estuarine communities. Von Holle and Ruiz (1997) document the negative effects of *Cordylophora caspia* on ciliates and bryozoa, while the hydroid attracted barnacles, amphipods and polychaetes. They suggest that this hydroid is a major restructuring force in the fouling community of Baltimore Harbor, Maryland.

*Cordylophora* spp. also occurs with the zebra mussel, *D. polymorpha*, at locations in Europe (Jenner and Janssen-Mommen 1993; Moreteau and Khalanski 1994) and in Illinois (J. Stoeckel, pers. comm.; pers. obs.). Competition for space may occur between the hydroid and *D. polymorpha* (Smit *et al.* 1993; Walton 1996). The presence of *Cordylophora* spp.

may enhance or facilitate recruitment of *D. polymorpha* (Dean and Hurd 1980; Moreteau and Khalanski 1994; M. Khalanski, pers. comm.). The hydroid may also provide a physical barrier to *D. polymorpha* settlement and/or may act as a larval filter (Standing 1976). Lastly, filtering by clumps of *D. polymorpha* may provide a more favorable substrate for *Cordylophora* spp. by enhancing flow. This type of symbiotic relationship is similar to associations between sponges and brittle stars in the Caribbean (Hendler 1984). Perhaps some ecological association exists between these organisms that enhances their roles in biofouling (Walton 1996). This is yet to be investigated.

#### FUTURE RESEARCH

With the range of *Cordylophora* spp. expanding, it is of great interest to identify the factors responsible for this expansion. Future research with populations of *Cordylophora* spp. should address the following: (1) global population locations and physiochemical water conditions at each population location, (2) hydroid morphometric features such as colony height and polyp morphology, (3) genetic similarities and differences among populations collected, and (4) interbreeding capabilities among populations sampled.

As mentioned previously, *C. caspia* and *C. lacustris* are considered conspecifics though the existence of different species is possible. The species names *caspia* and *lacustris* have been used interchangeably without clear scientific documentation of anatomical or genetic differences for various *Cordylophora* spp. populations. To ascertain whether different species exist, morphological and genetic data need to be carefully analyzed.

Distinguishing morphological and genetic differences in populations of *Cordylophora* spp. will provide information to trace invasion routes and help explain the range expansion of *Cordylophora*. It is of great interest to aquatic biologists to understand reasons for a given organism's range expansion, since it may become a larger fouling problem for power companies and may have a major impact on community structure in freshwater and estuarine ecosystems.

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