

**Rapid Communication****First record of the encrusting bryozoan *Cribrilina (Juxtacribrilina) mutabilis* (Ito, Onishi and Dick, 2015) in the Northwest Atlantic Ocean**

Thomas J. Trott\* and Claire Enterline

Maine Coastal Program, Department of Marine Resources, 194 McKown Point, West Boothbay Harbor, Maine, USA

Author e-mails: [tom.trott@maine.gov](mailto:tom.trott@maine.gov) (TT), [claire.enterline@maine.gov](mailto:claire.enterline@maine.gov) (CE)

\*Corresponding author

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**OPEN ACCESS****Abstract**

The cribrimorph bryozoan *Cribrilina (Juxtacribrilina) mutabilis* (Ito, Onishi and Dick, 2015), originally described from Hokkaido, Japan, is reported for the first time in the Gulf of Maine and the whole of the Northwest Atlantic coast. In September 2018, numerous colonies of *C. mutabilis* were collected from eelgrass (*Zostera marina* Linnaeus, 1753) beds near Clapboard and Mackworth Islands, and Hog Island Ledge, all located within inner Casco Bay, Maine. Bryozoan colonies encrusted eelgrass, rockweed (*Ascophyllum nodosum* (Linnaeus) LeJolis, 1863), and laminarian drift algae. Situated near the discovery location, the city of Portland (Maine, USA) is an active seaport, suggesting introduction through shipping as a likely introduction mechanism. The North Sea is hypothesized to be the most probable area for the source population. Since *C. mutabilis* appears to have high potential for introduction, it likely occurs on other parts of the Northwest Atlantic coast where it has yet to be identified and recorded.

**Key words:** *Zostera marina*, introduced species, Cribrilinidae, zooids, Casco Bay, Gulf of Maine

**Introduction**

The encrusting bryozoan *Cribrilina (Juxtacribrilina) mutabilis* (Ito, Onishi and Dick 2015) (Cribrilinidae) was discovered during routine sampling of eelgrass (*Zostera marina*) in Casco Bay, Portland, Maine, U.S.A. This species was originally described from collections made in eelgrass beds (*Zostera marina*) within Akkeshi-ko estuary, Hokkaido, northern Japan (Ito et al. 2015). There are no published reports of this species in the Northwest Atlantic, despite extensive reviews of the bryozoan fauna of the northeast coast of the United States (Virginia to Maine) from museum collections and field surveys (Winston et al. 2000; Winston and Haywood 2012).

*Cribrilina mutabilis* forms light pink, flat, small (5–7 mm across) circular encrusting colonies which contain three kinds of zooids, the frequency of each varying with season in Japan (Ito et al. 2015). An obligate eelgrass epibiont in Akkeshi-ko estuary (Ito et al. 2015), this species can occupy other substrata such as furoid and laminarian algae beyond its Hokkaido distribution (Ito et al. 2015).

While the native range of *C. mutabilis* appears to be unclear at this moment, Ito et al. (2015) hypothesized the origin of this species to be the northeastern Asian coast. Elsewhere in the Pacific Ocean, a single dead colony of this species was identified among the bryozoans in Japanese tsunami debris washed ashore on the Northwest American Pacific coast, specifically Washington state (McCuller and Carlton 2018). In the Atlantic Ocean, other than the discovery reported here, the only other records are from the North Sea: Norway (A Waeschenbach, Natural History Museum, London, *unpublished data*) and the west coast of Sweden (Ito et al. 2015).

This account provides a brief description of *Cribrilina mutabilis* followed by preliminary ecological observations discussed mainly in the context of those reported for Akkeshi-ko estuary populations.

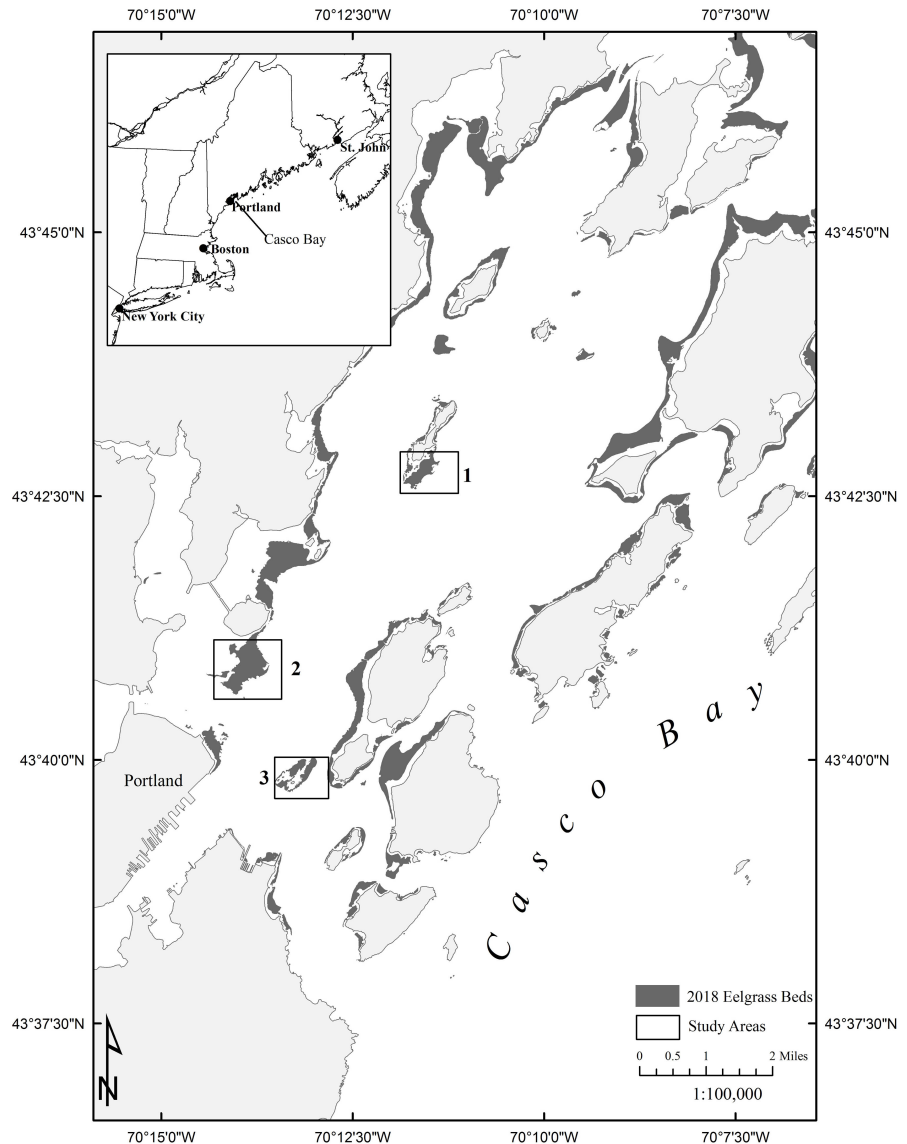
### Materials and methods

Eelgrass (*Zostera marina*) beds in Casco Bay, located off Portland (43.6591°N; 70.2568°W) on the southern coast of Maine (Figure 1), were surveyed on 12 September 2018. They were qualitatively sampled at depths of 2.33 to 4.76 meters approximately 2 h after low tide using a 0.05 m<sup>2</sup> Ponar grab sampler equipped with a GoPro video camera. Water column environmental parameters were measured with an EXO-1 YSI sonde attached to the grab sampler platform. The platform remained stationary on the bottom for at least 10 min before retrieval.

Collected macroalgae, both drift and attached, plus blades of live eelgrass, many with degenerating tips, were cut into smaller pieces (~ 3 cm) to make microscopic observations manageable. Sections with colonies were preserved in 95% ethanol within 24 h of collection. Cobble and shell were examined for the presence of colonies. Specimens were initially identified by T. Trott, and voucher specimens were sent to and confirmed by Dr. Matthew Dick, Hokkaido University, Japan.

Bryozoan incidence among eelgrass beds was estimated from video frames using Photoshop CC®. Incidence was defined as the ratio of number of blades with colonies versus without colonies. Frames were selected based on best water clarity which maximized visibility for counting colony occurrence and number of eelgrass blades within a masked focal area measuring 41% ± 0.04 (mean percent ± SD) of total image size (67.73 × 31.8 cm, resolution 28.346 pixels/cm). One frame was chosen from each video for each sample location. However, because of poor water clarity at two sample locations (EG005, EG007), a total of ten out of 14 videos were analyzed. All blades were counted, not plants, i.e., blades were not distinguished from originating out of a common shoot.

The frequency of zooid types and the proportion of reproductive zooids were determined using light micrographs. For each colony, zooid type was recorded for 10 randomly selected zooids, each color coded using Image-Pro®



**Figure 1.** Casco Bay, Maine, U.S.A. showing locations where *Cribrilina (Juxtacribrilina) mutabilis* was collected from eelgrass (*Zostera marina*) beds. Inset illustrates Casco Bay in relation to the Gulf of Maine. Locations: 1, Clapboard Island; 2, Mackworth Island; 3, Hog Island Ledge. Location numbers are the same as those in Tables 1 and 2. Map by C. Enterline.

to avoid re-examination. This procedure was repeated for 20 randomly selected colonies. The number of zooids containing embryos in each 10-zooid sample was also counted.

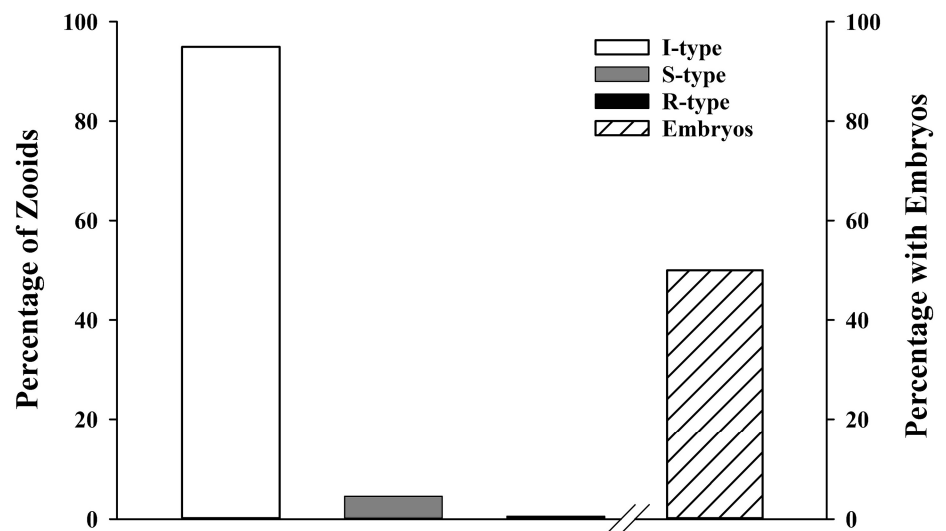
Voucher specimens were deposited in the Yale Peabody Museum (YPM IZ 103912, mixed zooid types, Clapboard Island; YPM IZ 103913, mixed zooid types, Mackworth Island; YPM IZ 103914, mixed zooid types on laminarian drift algae, Clapboard Island) and the National Museum of Natural History (USNM 1568351, mixed zooid types, Clapboard Island).

## Results

The Ponar grab was effective at sampling the bottom surface; however, depth of penetration was not consistent among eelgrass beds because of



**Figure 2.** Colonies of *Cribrilina (Juxtacribrilina) mutabilis* encrusting blades of eelgrass (*Zostera marina*) at eelgrass bed 1 near Clapboard Island, Casco Bay, Maine, U.S.A. Red arrows point to representative colonies. Photo by T. Trott.



**Figure 3.** Composition of colonies of *Cribrilina (Juxtacribrilina) mutabilis* per zooid type and occurrence of zooids with embryos. Percentages based on a total of 120 zooids selected among 12 colonies collected on eelgrass (*Zostera marina*) in Casco Bay, Maine, U.S.A. Zooids and colonies were randomly chosen for examination.

varying densities of roots and shoots. Eelgrass blades comprised the bulk of all samples, but drift algae and attached young rockweed (*Ascophyllum nodosum*) plants (3 cm maximum length) were also collected along with some shell and cobble.

Hundreds of colonies of the bryozoan *Cribrilina (Juxtacribrilina) mutabilis* were collected, and all eelgrass beds were colonized. Colonies were clearly visible in video recordings which showed *C. mutabilis* located particularly on the brown non-living distal ends of eelgrass blades (Figure 2). Colonies contained mostly I-type zooids with embryos, although some colonies had few R-type and S-type zooids (Figure 3). Because grab sampling was not

**Table 1.** Occurrence of *Cribrilina (Juxtacribrilina) mutabilis* among eelgrass (*Zostera marina*) beds in Casco Bay, Maine U.S.A. Incidence is reported as the mean of ratios of the number of eelgrass blades with colonies versus without colonies among samples for each location based on video frames recorded on 12 September 2018.

Bed No. <sup>a</sup>	Location <sup>b</sup>	Place Name	Incidence	SD	N	No. Blades <sup>c</sup>
1	43.71222°; -70.19278°	Clapboard Island	0.93	0.42	4	196
2	43.68167°; -70.23056°	Mackworth Island	0.67	0.36	3	119
3	43.66417°; -70.21972°	Hog Island Ledge	0.40	0.09	3	70

<sup>a</sup>Bed number corresponds to locations shown in Figure 1.

<sup>b</sup>Coordinates for the center of each location.

<sup>c</sup>Total number of eelgrass blades used in ratio calculations.

SD: standard deviation, N: total number of frames.

**Table 2.** Environmental conditions ( $\bar{x} \pm SD$ ) at eelgrass (*Zostera marina*) beds sampled in Casco Bay, Maine, U.S.A. where *Cribrilina (Juxtacribrilina) mutabilis* was collected.

Bed No. <sup>a</sup>	Sample	ODO (mg/L)	Temp (°C)	Salinity (ppt)	Chlorophyll (µg/L)	Depth (m)	N <sup>b</sup>
1	EG001	7.58 ± 0.01	17.01 ± 0.01	35.03 ± 0.01	1.71 ± 0.12	2.33	115
1	EG002	7.57 ± 0.02	16.9 ± 0.02	35.08 ± 0	2.15 ± 0.16	2.70	49
1	EG003	7.73 ± 0	16.94 ± 0	35.06 ± 0	2.38 ± 0.1	2.43	68
1	EG004	7.71 ± 0	16.88 ± 0	35.1 ± 0	2.35 ± 0.02	2.98	9
2	EG005	7.24 ± 0	16.71 ± 0	34.24 ± 0.01	2.05 ± 0.08	2.55	63
2	EG006	7.3 ± 0.01	16.59 ± 0	34.31 ± 0.02	1.41 ± 0.05	3.74	177
2	EG007	7.2 ± 0.01	16.86 ± 0.01	34.66 ± 0.02	2.19 ± 0.19	3.59	122
2	EG008	7.28 ± 0	16.61 ± 0.02	34.4 ± 0.01	1.55 ± 0.1	4.21	77
2	EG009	6.88 ± 0.05	16.79 ± 0.01	34.57 ± 0.02	1.71 ± 0.11	4.18	85
3	EG010	7.65 ± 0.01	16.36 ± 0	34.69 ± 0	1.92 ± 0.12	3.76	256
3	EG011	7.51 ± 0.01	16.27 ± 0	34.81 ± 0	1.27 ± 0.03	4.10	48
3	EG012	7.61 ± 0	16.28 ± 0	34.81 ± 0	1.68 ± 0.22	4.76	217

<sup>a</sup>Bed number corresponds to locations shown in Figure 1.

<sup>b</sup>N: number of iterations each parameter was measured at depth (m) approximately 2 h after low tide.

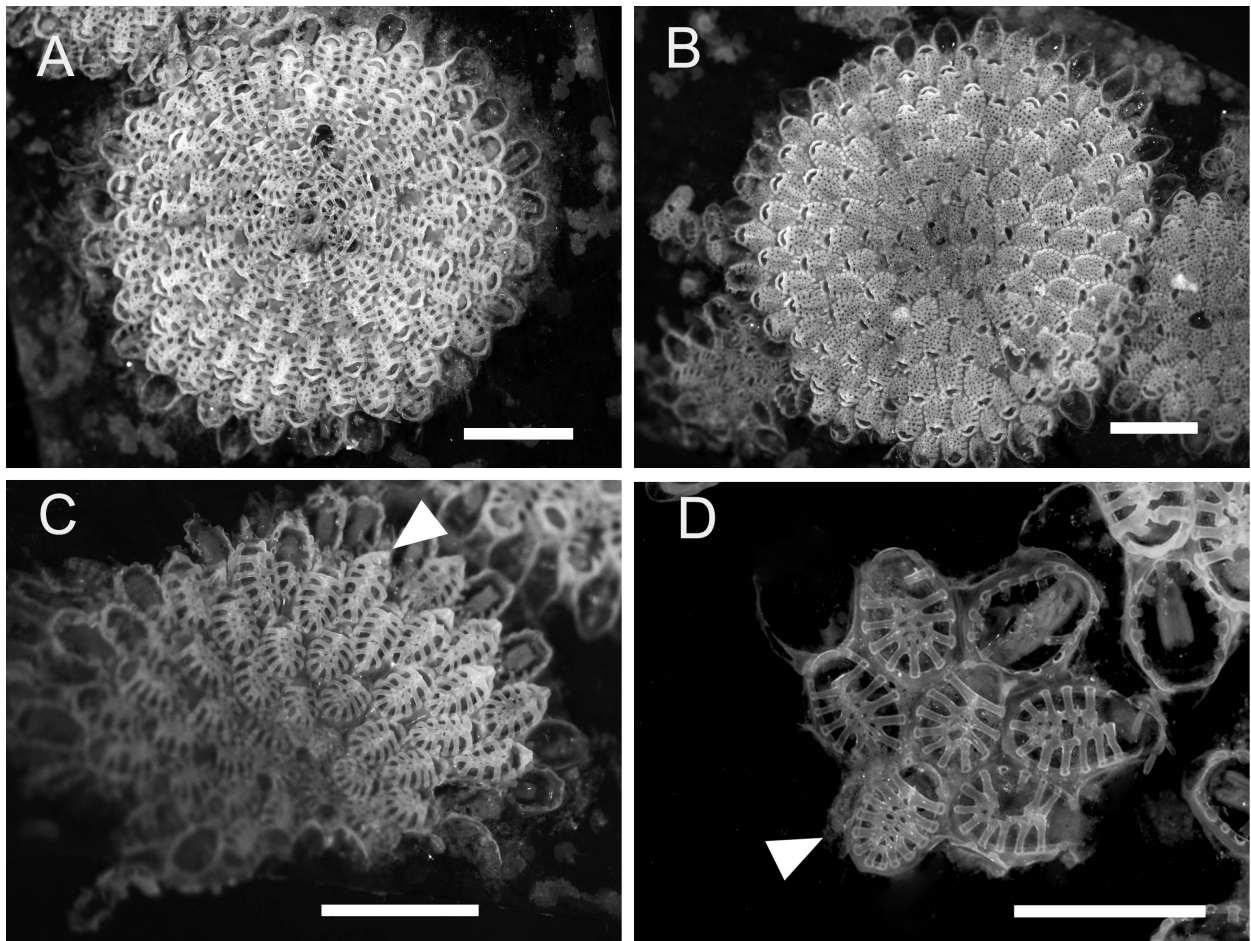
quantitative, video analysis gave better estimates of bryozoan occurrence among beds. Incidence of *C. mutabilis* was greatest at Clapboard Island, lowest at Hog Island Ledge, with Macworth Island between those extremes (Table 1). Colonies were also found on drift algae, but only at Clapboard Island. Owing to degradation, drift algae could not be identified beyond being of a laminarian type. New recruits, both single ancestrulae and small unidirectional colonies were present on eelgrass from Clapboard and Mackworth Islands and *Ascophyllum nodosum* from Hog Island Ledge. No colonies were found on shell or cobble.

With the exceptions of chlorophyll content and depth, water column environmental parameters varied little among eelgrass beds. Eelgrass bed 1, Clapboard Island, where the incidence of colonization was greatest, was the shallowest (2.33–2.98 m) and had the highest sea water temperature and dissolved oxygen concentration at depth (Table 2).

### *Specimen description*

General diagnosis (terminology adapted from Ito et al. 2015): colony encrusting, usually one layer of zooids, roughly circular in form, approximately 5–7 mm across; whitish-pink in color when live, lacking avicularia; zooids oval, distinct, with two to three distal spines; spinocyst highly convex, composed





**Figure 4.** Zooid types of *Cribrilina (Juxtacribrilina) mutabilis* collected from eelgrass beds in Casco Bay, Maine, U.S.A. (A) I (intermediate) type (25x). (B) S (shield) type (20x). (C) Mixed I and R (rib) types (30x). Arrow head points to R type zooid. (D) Early unidirectional colony (45x). Arrow head points to I type ancestrula. Scale bars: (A–C): 1mm; (D): 0.5mm. Photos are light micrographs of 90% ethanol preserved colonies. Photos by T. Trott.

of hollow, non-articulated costae resembling ribs with degree of lateral fusion dependent on zooid type; live embryos yellow-orange.

Zooids are of three types:

*I (intermediate) type (Figure 4A)*

Spinocyst with costae more numerous and narrower than R type, with varying degree of distal and lateral fusion creating elliptical shield of variable size with scattered intercostal pores.

*S (shield) type (Figure 4B)*

Spinocyst appearing as shield from distally fused costae; intercostal pores with radial arrangement; basal openings narrower than costae, size reduced; costae of orifice apertural bar thickest, separate from adjacent costae by gap from basal opening and at least one lumen pore.

*R (rib) type (Figure 4C)*

Spinocyst with costae widely spaced, large basal openings, fused at midline, appearing like vertebrate rib cage. No intercostal pores.

Early colonies unidirectional, initially formed by distal bud of ancestrula followed by paired distolateral budding (Figure 4D).

A more complete description of zooid types and distinguishing morphological characteristics is given in Ito et al. (2015).

## Discussion

The discovery of *C. mutabilis* in Casco Bay, Maine, is the first for the Northwest Atlantic Ocean. Three other species of *Cribrilina* (none of which were found in our current Casco Bay samples) occur regionally, two with ostensible amphi-Atlantic distributions (*C. annulata* (Fabricius, 1780) and *C. cryptoecium* Norman, 1903) and one (*C. macropunctata* Winston, Hayward and Craig, 1999) described only from the West Atlantic coast (Winston et al. 2000). Among other characters, the presence of three zooid types distinguishes *C. mutabilis* from these congeners. The presence of solitary ancestrulae and young unidirectional colonies indicates recruitment within this population and rules out the possibility its occurrence was due to chance of a single settlement event.

Colonies were mostly on the brown non-living distal ends of eelgrass blades, an arrangement observed *in situ* and in the laboratory. Colonies were also found on brownish dead or dying blades of drifting *Z. marina* near Chikarakotan, Akkeshi-ko estuary (Ito et al. 2015). *Zostera marina* produces phenolic acids, chemicals which, in the laboratory, reduce grazing and establishment of some epifaunal species (Harrison 1982a, b). Such compounds could explain why colonies were mostly found on dead or dying portions of living eelgrass. However, a more likely explanation is that colonies persisted beyond the lifetime of the sections of eelgrass blades where they became established.

The types of substrata on which *C. mutabilis* occurs varies with geographic location, an observation that supports the idea of population preferences for substratum selection during larval settlement. In Akkeshi-ko estuary and adjoining Akkeshi Bay, colonies were found on *Zostera marina*, and not discovered during casual searches of other seagrasses (*Z. japonica* Ascherson and Graebner, 1907, *Z. asiatica* Miki, 1932, and *Phyllospadix iwatensis* Makino, 1931) (Ito et al. 2015). However at Kristineberg, Sweden, colonies were found by AN Ostrovsky, University of Vienna, on *Zostera* sp., *Laminaria* spp., and *Fucus* spp. (as reported in Ito et al. 2015). Similarly, in Casco Bay, Maine, colonies were found on *Zostera marina*, laminarian drift algae, and the furoid *A. nodosum*. Occurrence of colonies on hard substrata is less well known. In Akkeshi-ko estuary and adjoining Akkeshi Bay, *C. mutabilis* was not detected among rocky intertidal habitats (Grischenko et al. 2007; Ito et al. 2015). However, hard substrata may be used by populations occurring on the northeastern Asian coast if the suggestion by Ito et al. (2015) is correct; that some specimens from that region identified

as *Membraniporella* (synonymized with *Klugerella*) *aragoi* Audouin, 1826 were, in fact, *C. mutabilis*. *Klugerella aragoi* was found on mollusc shells, wharves, fishing boats and acrylic settling plates (Kubanin 1975). Unknown is whether *C. mutabilis* occurs on hard substrata in Casco Bay, Maine, and Kristineberg, Sweden since these surfaces have yet to be extensively explored. Cobble and shells from eelgrass beds sampled in Casco Bay had no established colonies, but the amount of material examined was very limited. Substrate specificity has important consequences for potential dispersal mechanisms, such as rafting, and may also characterize differences in ecology among geographically separated populations of *C. mutabilis*.

The zooid types of *C. mutabilis* are discrete and do not represent gradual transitions of individual zooids from one type to another, a feature shown by Ito et al. (2015) who observed a phenology in the development of different zooid types with season. Casco Bay colonies contained mostly I-type zooids with embryos, although a few R-type and S-type zooids were also present. In Japan during August, I-type zooids predominate among colonies collected from Akkeshi-ko estuary (Ito et al. 2015). The dominance of I-type zooids among colonies collected from such geographically distant populations is notable, since season (late summer), sea water temperature ( $\sim 17\text{ }^{\circ}\text{C}$ ), and latitude are similar for collections from Casco Bay and Akkeshi-ko estuary. In contrast, the proportion of zooid types diverged among colonies collected at different latitudes, i.e., Akkeshi-ko ( $43^{\circ}\text{N}$ ) versus Kristineberg ( $58^{\circ}\text{N}$ ), but during the same month (June) (Ito et al. 2015). These observations suggest that colony composition is influenced by sea water temperature, notwithstanding other influencing factors. Although the over-wintering zooid type is not known, Ito et al. (2015) suggested it could be nonreproductive S type zooids since these were observed in late August and their structure offers more physical resistance to winter conditions. They hypothesized that the production of different zooid types may represent an energy trade-off between what is required for reproduction and  $\text{CaCO}_3$  production.

The spatial extent and number of eelgrass beds sampled were too limited to permit generalizing about how the spatial distribution of *C. mutabilis* relates to environmental conditions in Casco Bay. A more exhaustive quantitative survey that maps the distribution of *C. mutabilis* in the Bay could yield a predictive model for its occurrence based on environmental conditions. However, comparisons of the physical parameters characterizing eelgrass habitats at collection sites in Casco Bay and Akkeshi-ko estuary give some insight into the physiological tolerances of *C. mutabilis*. This species can live in a wide range of salinities varying from brackish to full seawater, with Akkeshi-ko estuary (6.2 to 27.6 ppt; Ito et al. 2015) and Casco Bay (approximately 25–35 ppt; Casco Bay Estuary Partnership 2015) at extremes of the known range. Salinity at Kristineberg Marine Research



Station, Sweden, near where this species was found in the North Sea, ranged from approximately 20.81 to 33.26 ppt in 2017 (KMRS 2018). Turbidity can vary greatly where *C. mutabilis* is found, particularly since eelgrass beds in Japan are established in sandy sediments while Casco Bay beds are in muddy-sand (Maine Coastal Program, *unpublished data*). Sediment suspension would be greater in Casco Bay where wave exposure and 2.78 m average tidal range (NOAA 2018) is greater than for the sheltered estuary of Akkeshi-ko where the tidal range is 1.57 m (Ito et al. 2015). In summary, *C. mutabilis* appears to be euryhaline and very adaptable to varying environmental conditions. These features indicate a high potential for successful introduction.

Relative to how *C. mutabilis* was introduced to Casco Bay, Portland is the largest tonnage seaport in New England, a major U.S. east coast seaport, and the largest foreign inbound transit tonnage port in the United States (Acheson and Acheson 2016). This species may thus have arrived through commerce with container ships originating from the North Sea (McGuire 2019). However, in view of its high potential for successful introduction, *C. mutabilis* likely occurs on other parts of the Northwest Atlantic coast and has yet to be identified and recorded. If that is the case, an introduction into Casco Bay could have occurred from more regional Northwest Atlantic populations. Molecular analysis now underway may help reveal one or more source populations.

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