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Article

Idotea balthica comparison: Anatomy, locomotion, and seaweed preference of Massachusetts isopods

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SUMMARY

We investigated a select population of Massachusetts marine isopods to determine if they are of the species *Idotea balthica* by comparing their features to those of *I. balthica* living in the Baltic Sea. We hypothesized that the Massachusetts isopods belonged to the species, despite the large geographical distance between the two populations. For this study, a group of 12-18 isopods were captured in Plymouth, Massachusetts in October 2020 and observed in captivity. After examination, they were then compared in three areas (morphology, movement, and seaweed preference) using previous studies on the Baltic Sea isopods as references for comparison. Results indicated that the Massachusetts isopods resembled the Baltic Sea isopods in most areas, with the few key exceptions of smaller female length, slightly darker coloration of males, and greater seaweed preference for *Chondrus crispus* with no inclination toward *Pilayella littoralis*. This knowledge could be useful for future examination of the two groups, such as when measuring their responses to climate change, especially given the physical and ecological differences between the Massachusetts Bay and Baltic Sea marine environments.

INTRODUCTION

As climate change is altering marine ecosystems worldwide, identifying local species and studying geographically distant populations within a species can be a tool for understanding the effects of climate change on different ecosystems. One such species with an extensive geographic range is the marine isopod *Idotea balthica*. These small crustaceans represent key components of the food webs in the temperate intertidal communities they inhabit, as both grazers of benthic seaweed and prey for many other marine species. A deepened understanding of the less-documented *Idotea* populations in areas such as the eastern coast of North America could be useful for studying how the species adapts to human disruption and climate change. In addition, examining intraspecific variability in *Idotea* could help to illuminate any distinct roles it may play in the varied ecosystems of its geographic distribution.

While research has been conducted on *I. balthica* in the European parts of the North Atlantic, the populations in New England are less extensively studied (1, 2). Notably, a comprehensive study on *I. balthica* in the Baltic Sea was previously conducted by Leidenberger *et al.* (1). The species *I. balthica* is a marine crustacean belonging to the order Isopoda, a large and diverse clade encompassing terrestrial, freshwater, and saltwater species of varying sizes (3). *I. balthica* and its close relatives inhabit bodies of water including the Baltic Sea and the North Atlantic, where they have been found along the North American east coast from Nova Scotia to as far south as Virginia and in Europe from Norway's west coast to the French side of the English Channel, as well as in Iceland (4, 5). Members of this species can exhibit wide variation in color but are generally green to brownish-tan in color and superficially resemble insects (5). They exhibit sexual dimorphism, with organisms measuring up to about 22 mm in length for males and about 15 mm for females (1).

As detailed by Leidenberger *et al.*, *I. balthica* occupies an important place in the littoral ecosystem as an omnivorous grazer that feeds on both microalgae and macroalgae, decomposing organic material, and lesser invertebrates (1). Previous to our study, Leidenberger *et al.* observed that isopods of this genus prefer certain seaweed types for both habitation and consumption, most notably *Fucus vesiculosus*, *Zostera marina*, and *Pilayella littoralis* (1). Orav-Kotta and Kotta noted that when given a choice, isopods preferred *F. vesiculosus* for habitat but *P. littoralis* for consumption (2). In addition to habitats and food sources, these seaweed species provide isopods with shelter from their numerous predators, mainly cod, perch, decapod shrimps, and flatworms (1).

In this study, we sought to characterize an unidentified isopod population native to Plymouth, Massachusetts, which bore the closest resemblance to the genus *Idotea*. We hypothesized that this isopod population belonged to the species *I. balthica*, which appeared to be the closest match in terms of anatomy. We also examined five other North Atlantic *Idotea* species, specifically *I. chelipes*, *I. emarginata*, *I. granulosa*, *I. metallica*, and *I. pelagica* as comparators for anatomy. Comparison with additional data of the known *I. balthica* residing in the Baltic Sea aided our classification of the Massachusetts population. It would be a noteworthy development if the studied population were found to not belong to *I. balthica* or any of the other compared species, since the Massachusetts isopods could then theoretically represent a novel species. However, our results indicated that the New England population did belong to the expected species, while exhibiting minor intraspecific variations in length of females, coloration of males, and seaweed inclination.

RESULTS

Three categories were used to examine the Massachusetts *Idotea* population: anatomy, locomotion, and seaweed preference. By analyzing these various aspects, we aimed to better understand whether the isopods represented a novel population of the previously documented *I. balthica* species, belonged to a related species of *Idotea*, or belonged to a different species altogether.

Anatomy

The results of the anatomical comparison showed that the Massachusetts specimens exhibited predominantly similar characteristics to known examples of *I. balthica* **(Table 1 and Figure 1)**. When compared with other candidate species, the Massachusetts specimens' features aligned most closely with *I. balthica* **(Table 1)**. The Baltic Sea and Massachusetts females were both observed to be dark brown in color (1). Not counting antennae length, the Massachusetts isopods had maximum lengths of 21.2 mm out of three males measured and 10.0 mm out of three females measured, while *I. balthica* measured up to 21.9 mm for males and 14.8 mm for females (1). Despite the difference observed between Massachusetts and Baltic Sea females, *I. balthica* was still the closest match in length out of the six candidate species. The other species were all significantly shorter or longer than the Massachusetts isopods (1, 6, 7, 8). In perhaps the clearest identifying characteristic, the Massachusetts specimens displayed the same sharp tridentate shape of the telson, or tail segment, unique to *I. balthica* but not its close relatives (1, 6, 7, 8). Both the Baltic Sea and Massachusetts specimens were observed to have seven visible pereonites, or thorax segments, for both males and females (1).

Figure 1: Comparison of deceased Massachusetts *Idotea balthica* (on left) and Baltic Sea individuals (on right). The Massachusetts specimens shown were the largest of the group under observation. Note that the male's antennae are dried together, and the female's antennae are not visible. The picture of the Baltic Sea specimens is taken from Leidenberger *et al.* (1).

However, the Massachusetts isopods did exhibit several differences from *I. balthica*. One such difference was the darker yellowish-brown coloration seen in Massachusetts males **(Figure 1)**. The females were also smaller, with the Massachusetts females being 32% shorter than their Baltic

	MA specimens	*I. balthica**	*I. chelipes**	*I. emarginata*†	*I. granulosa**	*I. metallica*‡	*I. pelagica*^
Male color	Dark yellow	Pale yellow	Pale yellow	Golden yellow	Dark yellow	Grayish brown	Brown with pale central stripe
Female color	Dark brown	Dark brown	Dark brown with tan central stripe	Dark brown with pale yellow border	Reddish brown	Grayish brown	Dark brown with pale central stripe
Male length (max)	21.2 mm	21.9 mm	17.3 mm	30 mm	16.7 mm	30 mm	11 mm
Female length (max)	10.0 mm	14.8 mm	13.0 mm	18 mm	12.6 mm	18 mm	10 mm
Telson shape	Sharply tridentate, middle projection longest	Sharply tridentate, middle projection longest	Three shallow rounded projections, middle longest	Depression in middle, sharply pointed corners	Elongated rounded central projection, sloped sides	Broad, paddle-shaped, horizontal edge	Blunt point, curved sides
Number of pereonites	7	7	7	7	7	6	7

Table 1: Qualitative and quantitative anatomical characteristics of North Atlantic *Idotea* species. Reported male and female lengths represent the maximum values observed for each species, with the number of significant figures in each value retained from its source.
*The data for *I. balthica*, *I. chelipes*, and *I. granulosa* are from Leidenberger *et al.* (1).
†For *I. emarginata*, the data for male color, female color, telson shape, and number of pereonites are from Fenwick, and male and female length are from Tyler-Walters (12, 6).
‡For *I. metallica*, the data for male color, female color, telson shape, and number of pereonites are from Lazo-Wasem and the data for male and female length are from the British Myriapod and Isopod Group (13, 7).
^For *I. pelagica*, the data for male color, female color, telson shape, and number of pereonites are from Hillewaert and male and female length are from Tyler-Walters (14, 8).

counterparts and 52% shorter than the males, as opposed to Baltic females being 32% shorter than Baltic males **(Table 1)**. This led to a disparity in the female-to-male size ratio, which was found to be 1:2.1 maximum as opposed to the 1.48:2.19 reported by Leidenberger *et al.* for *I. balthica* in the Baltic Sea (1).

Throughout the study, we also observed all organisms to increase rapidly in size from the time they were captured to when the study concluded. This could have been due to reduced competition over food, whether from sparser *I. balthica* population density, the absence of other species, or an increased supply of the food itself. Their growth was unanticipated, especially given the prediction of weakened growth due to the stress of being in an artificial environment.

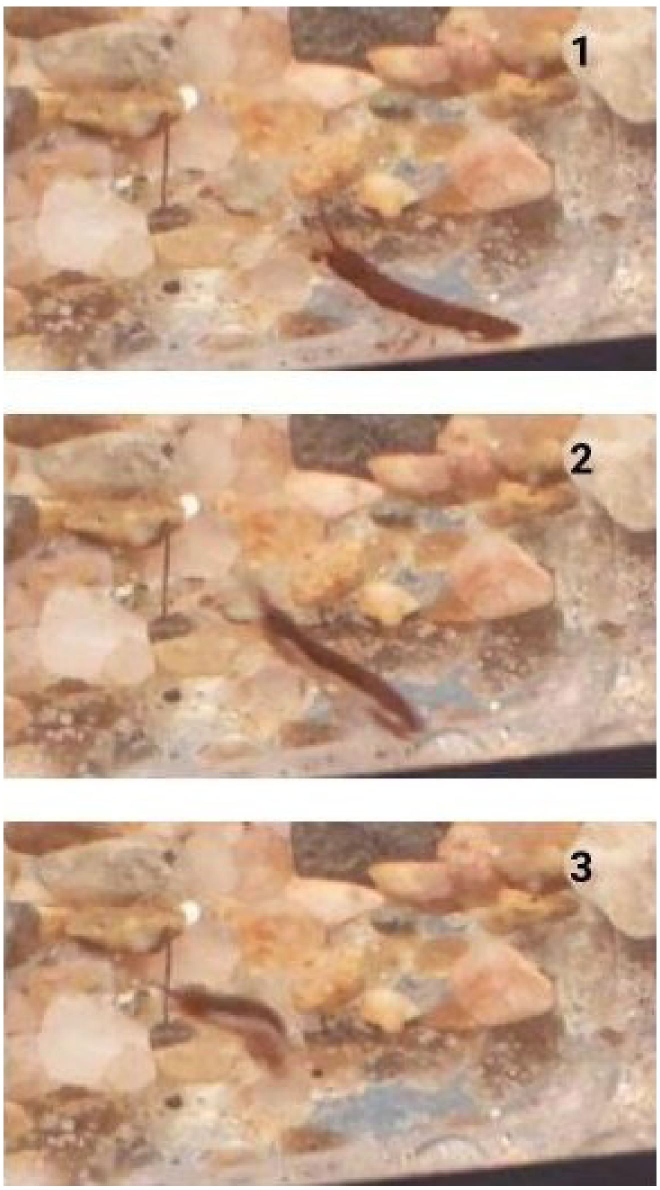

Figure 2: Locomotion of a female Massachusetts isopod. The series of freeze frames captures one propulsive stroke, consisting of tail extension and flexion. Frames are numbered in chronological order.

Locomotion

We found that the Massachusetts isopods swam with a jerky up-and-down motion of the tail, which appeared to provide their primary means of propulsion **(Figure 2)**. The legs did not seem to play a significant role in swimming, but all sets of limbs were used to support weight when walking on a surface. Individuals were frequently observed swimming freely, particularly the males, which fits previous descriptions of the isopods' search for food sources and mates (1). This behavior also makes sense in light of *I. balthica*'s niche as the species of *Idotea* that most frequently swims in open water farther from shore (1).

Seaweed Preferences

We looked at five commonly found local seaweed species when investigating isopod seaweed preference. First, we chose *Fucus vesiculosus,* a brown alga with distinctive air bladders, for its widely documented relationship with *I. balthica* as a source of both food and habitat (1). Second, we selected *Pilayella littoralis*, an often epiphytic filamentous brown alga, for its recorded use as a food source by isopods and a preferred habitat when growing on *F. vesiculosus* (2). Third, we used *Zostera marina*, a ubiquitous seagrass species, as it has also been documented as a habitat source for *I. balthica* (1, 2). Finally, we included for experimental comparison two species common to the region but not strongly associated with *I. balthica*: *Chondrus crispus*, a fan-shaped red alga, and *Saccharina latissima*, a brown alga with wide, flattened blades.

We measured seaweed habitation preference rather than consumption. The Massachusetts isopods were most active in the evening and least active at midday, which roughly aligns with Leidenberger *et al.*'s findings about *I. balthica*'s activity levels during the autumn (1). We observed the following number of instances of occupation: 14 morning, 10 midday, and 24 evening for *C. crispus*; 19 morning, 16 midday, and 34 evening for *F. vesiculosus*; 0 morning, 0 midday, and 0

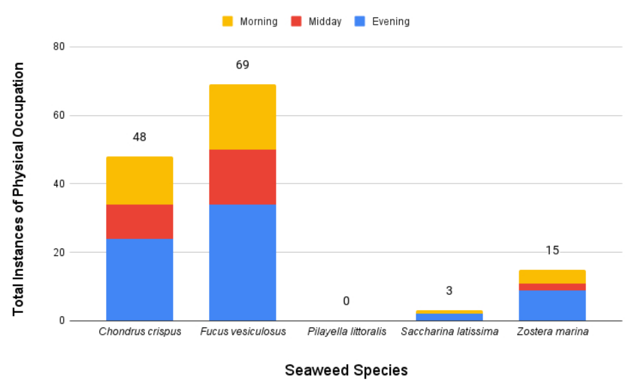

Figure 3: Seaweed perching preferences of the Massachusetts group. Above each column is the total number of instances an isopod was seen on each seaweed species. The totals were divided into the three separately recorded categories: morning (7:30 AM EST), midday (12:30 PM EST), and evening (5:30 PM EST).

evening for *P. littoralis*; 1 morning, 0 midday, and 2 evening for *S. latissima*; and 4 morning, 2 midday, and 9 evening for *Z. marina*.

In terms of general habitation frequency, the isopods displayed a strong inclination toward *F. vesiculosus*, which matches the findings of Leidenberger *et al*. and Orav-Kotta and Kotta **(Figure 3)** (1, 2). The isopods also tended toward *C. crispus* as their second most favored choice, showing a higher attraction to this seaweed type than has been recorded for those in the Baltic Sea (4). They perched on *Z. marina* only occasionally. In the wild, organisms have been observed rafting on free-floating blades of *Z. marina* in open water and sometimes eating epiphytes such as filamentous algae that grow on *Z. marina*, but they have rarely been documented using *Z. marina* itself as a food source (1). The Massachusetts isopods also infrequently visited *S. latissima*, which was expected since *Idotea* habitation and consumption have not been recorded for this species. However, throughout the entire experiment, we never observed isopods perching on *P. littoralis*. This represents a significant difference from Orav-Kotta and Kotta's findings, which indicate that *I. balthica* relies on *P. littoralis* as a key source of nutrition and may actually prefer it over *F. vesiculosus* for consumption (2).

DISCUSSION

This study aimed to classify a previously unidentified population of marine isopods from Plymouth, Massachusetts. After narrowing down candidate species to the genus *Idotea*, we studied a group of isopods from the Massachusetts population in captivity for approximately five weeks, examining their anatomy and comparing specific anatomical details with those of six species of *Idotea*. We also studied their means of locomotion and measured their inclination toward various seaweed types based on perching frequency. We compared these findings with the corresponding data on *I. balthica*, which the Massachusetts isopods most closely resembled in morphology. The data we found suggest that the unidentified Massachusetts isopods are of the same species as *I. balthica* from the Baltic Sea. Their general shape identified them as belonging to the genus Idotea, and their tridentate telson distinguished them further as belonging to *I. balthica*. Corroborating this classification, their overall length, coloration, and seaweed preference matched those previously described by Leidenberger *et al*., and they exhibited the swimming behavior expected of *I. balthica* (1).

While we concluded that the Plymouth Long Beach marine isopod population likely belongs to the species *I. balthica*, they did exhibit several differences from their Baltic Sea relatives. These differences include darker coloration seen in males, shorter length in females, and increased preference toward *C. crispus* while lacking interest in *P. littoralis* altogether, at least in captivity. These differences can likely be attributed to intraspecific variation. Grazing behavior and coloring, for instance, are characteristics that have been observed to vary between populations of *I. balthica* as well as between individuals in the same population (1, 4, 9). Alternately, *I. balthica* is known to be able to regulate their color saturation in order to blend in with changing light and color of their surroundings; however, it may be difficult to test whether the Massachusetts specimens' color variations are due to this phenotypic plasticity or due to genetic variation (1, 9). In the case of seaweed preference, either the isopod species or the algae species could be exhibiting intraspecific variation. Either is possible, since *C. crispus* especially has been noted to exhibit wide morphological variation in areas such as color, plant size, and frond width, all of which could contribute to increased or decreased attractiveness to isopods (10). Beyond intraspecific variation, the isopods' choice of seaweed could also have been influenced by factors related to being in captivity. For instance, stress from introduction to an unfamiliar environment could have caused them to seek out denser seaweed species that provide more shelter. Conversely, if there were fewer perceived predators, the isopods could have been more willing to perch on the exposed tops of fronds. However, it should be noted that the study's sample size of 12-18 organisms may not have been sufficient to accurately represent the full range of intraspecific variation within the Plymouth population, especially in the areas of length and coloration. We also theorized that perhaps we had misidentified the seaweed labeled as *P. littoralis* in the study of the Massachusetts population, which would have led to inaccurate results regarding their inclination toward *P. littoralis*. Future research would benefit from an expanded sample set and more rigorously verified seaweed identification, and potential research directions could entail looking for the occurrence of color regulation in Massachusetts isopods.

According to Wares' DNA analysis of *I. balthica* representatives from various North Atlantic populations, several genetically distinct North American clades exist (4). Given the significant barrier of the Atlantic Ocean, one would expect that the North American isopods would form one genetically related group, while the European isopods would belong to another. Yet as seen in Wares' work, the grouping has been shown to be more complex (4). Using phylogenetic modeling based on nucleotide sequences, a Rhode Island specimen was classified as most closely related to those from Nova Scotia, Maine, and France, as opposed to those from Iceland, Ireland, and Virginia, and a separate group found in France (4). The Massachusetts isopods investigated in this experiment are geographically closest to the Rhode Island specimen (4). If *I. balthica* has a large genetic pool and the Massachusetts and Baltic Sea populations are more distantly related to each other, then more variations could be expected in the Massachusetts specimens. Further research is needed to compare their physiological traits with those of possibly closer relatives, such as specimens from Nova Scotia or Maine.

As can be noted in Wares' study, Plymouth, Massachusetts is within *I. balthica*'s documented range, so it is not particularly surprising to discover them there (4). However, recording the species' presence in Massachusetts will be useful for adding to the catalog of known marine fauna in littoral New England communities. This also facilitates opportunities for intraspecific comparison between populations in the contrasting environments of the Baltic Sea and the Massachusetts Bay area, particularly when considering the differences in biodiversity and salinity between the two bodies of water. Furthermore, this knowledge could be helpful in the context of climate change, as monitoring the well-being of a population in a more severely impacted ecosystem like the Baltic Sea could provide insight into the future prospects of other populations, such as those in New England. Our results could also be of use in noting any potential differences in how geographically distant populations respond to the effects of climate change.

Further research might entail field study at Plymouth Long Beach to discern whether other *Idotea* species are present at other sections of the beach or in the nearby Plymouth Harbor. Behavioral topics of interest include additional study of their seaweed-rafting habits as well as interactions with other animal species, including humans. It would also be interesting to investigate potential reasons for the coloration differences between Massachusetts and Baltic Sea males, especially given the near-identical coloration of females.

MATERIALS AND METHODS

The 12-18 isopods studied were caught at Long Beach in Plymouth, Massachusetts, on October 18, 2020. Once at the author's home, they were transferred to a 50 x 25 x 42 cm glass fish tank filled to the 35-cm height mark with saltwater collected from Long Beach on the same day. Representatives of five common species of North Atlantic seaweed were also collected there, ensuring that these species were present in both North American and Baltic Sea waters for virtue of comparison. These were added to the tank, along with a small amount of sand. The isopods were then cared for and observed for 38 days, from October 18, 2020 to November 24, 2020. No outside material was introduced to the habitat until a large quantity of extra seaweed was added on November 22 in order to replenish their food supply. However, this action may have led to their death, which occurred two days thereafter, speculatively caused by anoxia or hydrogen sulfide released in the water by the seaweed's decomposition (11). For the integrity of results, the recorded data on seaweed preferences is taken only from before the November 22 addition of seaweed, which could have altered the isopods' choices and behavior.

Three properties of the isopods were examined: anatomical features, locomotion style, and preferred seaweed species for habitat and consumption. Their anatomy was documented using pictures of deceased specimens taken with a Google Pixel 3, with Leidenberger *et al.*'s photos of Baltic Sea specimens for comparison (1). The lengths of three males and three females were measured using a metric ruler, from the projecting central tip of the telson to the top of the cephalon, not including the antennae. Out of all lengths recorded, the maximum lengths observed for males and females were used for the comparison in order to be consistent with Leidenberger et al.'s methods (1). The isopods' movement was captured on video with the same device and then compared to Leidenberger *et al.*'s written descriptions (1). For determining seaweed preference, five North Atlantic seaweed species were used: *Chondrus crispus* (common name: Irish moss), *Fucus vesiculosus* (common name: bladderwrack), *Pilayella littoralis*, a filamentous brown alga, Zostera marina (common name: eelgrass), and *Saccharina latissima* (common name: sugar kelp). All seaweed was taken from Plymouth Long Beach, Massachusetts, on October 18, 2020. Approximately five to six free-floating samples of each seaweed species were introduced. It is worth noting that the amounts were not equal in mass. For example, four blades of *Z. marina* would have less mass than four fronds of *F. vesiculosus* due to the differing structures of the two species. However, the seaweed quantities used attempted to match what the isopods would have encountered in the wild, and thus this disparity in mass was not predicted to affect habitation choice.

The isopods were observed every day at 7:30 AM, 12:30 PM, and 5:30 PM EST, recording at each interval the raw number of isopods seen perched on each seaweed type. Exact amounts of time spent on the seaweed types were not monitored. It is conceivable that, for instance, the same isopod could have been on *C. crispus* in the morning and remained in the same place at noon, which would have then been recorded as two separate data points instead of one. It should be noted that the isopods' seaweed choices were measured only in terms of general preference for occupation, which meant the data did not necessarily indicate whether they were also using it for food or solely for habitat. The data collected was then compared with observations from Leidenberger *et al.* and Orav-Kotta and Kotta regarding the seaweed choices of *Idotea* in the Baltic Sea (1, 2).

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