Boffin Access
FOSTERING KNOWLEDGE

Open Access Journal | Volume: 1.1

Marine Science and Biology

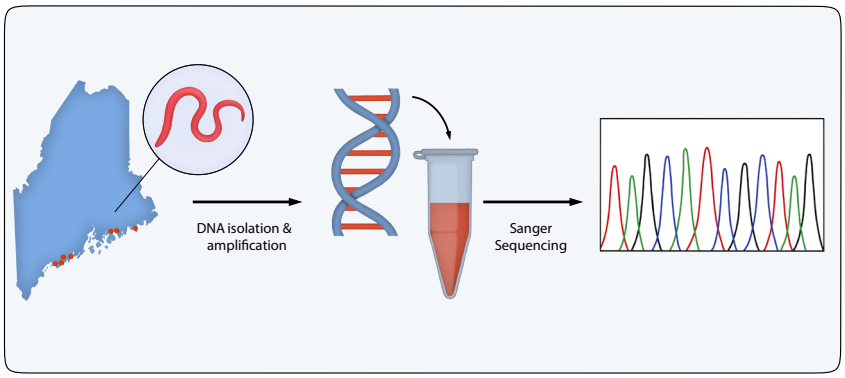
Bloodworm (*Glycera dibranchiata Ehlers,* 1868) Populations in the Gulf of Maine Are Connected Through Gene Flow

Larissa M. Williams[1]
Anna Marie Bowsher[1]
Maria-Anna Chrysovergi[1]
William G. Ambrose Jr.[2]

[1]Department of Biology, Bates College, USA
[2]Department of Coastal and Marine Systems Science, Coastal Carolina University, USA

Abstract

The polychaete bloodworm, *Glycera dibranchiata Ehlers*, 1868 is an important source of bait for saltwater fishing and is harvested from mud flats in the Gulf of Maine. Little is known about the life history of *G. dibranchiata*and management of the fishery is minimal. The goal of our study was to determine the population genetics of several Maine populations in order to inform management. We sequenced the neutral mitochondrial cytochrome c oxidase I (COI) marker in seven populations along the coast of Maine. A total of 486 nucleotides were sequenced in each individual, yielding 13 haplotypes, with 94% of them haploytpe 1. There was very little to no genetic differentiation among populations in the south (F_{ST} of less than 0.05), and there was only moderate differentiation between one of the most northern sites and the most southern site (F_{ST}between 0.05 and 0.15). This lack of genetic differentiation indicates the populations are genetically linked via gamete dispersal. Consequently, the bloodworm fishery in the State of Maine can be managed at the scale of the Gulf of Maine(Graphical abstract).



Graphical Abstract

Keywords

Bloodworm, *Glycera*, gene flow, cytochrome c oxidase I (COI), Gulf of Maine

Introduction

Polychaetes are harvested recreationally and commercially for fish bait worldwide [1]. Management of these fisheries is difficult because there is limited data on life histories of the target species and harvesting practices and the harvesters are notoriously difficult 60 to engage [2]. The bloodworm *Glycera dibranchiata Ehlers*, 1868 is one of the five most valuable (retail price per kg) marine species globally and one of the most popular polychaete species used as saltwater bait on the East Coast of North America [2].

Harvested from mud flats in Maine since the 1930s [3], bloodworms support a sustainable and large industry in the state with an average landed value of $6.3 million over the past 5 years [4]. Yearly fishing effort in Maine, as calculated by dividing the number of harvested pounds by the number of licenses 68 issued, has been relatively consistent [4] (Table 1), but there has been significant 69 variability in the catch from a recent high in 2002 of 682,994 lbs. to an unofficial low in 2018 of 376,294 lbs. Inter-annual variability in catch could be a consequence of market demand [5], but might also be the result of overharvesting in some areas. There is concern that overharvesting could become widespread if the fishery is not properly managed. Furthermore, climate change, which has led to the Gulf of Maine

Article Information

DOI: 10.31021/msb.20201101
Article Type: Research Article
Journal Type: Open Access
Volume: 1 **Issue:** 1
Manuscript ID: MSB-1-101
Publisher: Boffin Access Limited

Received Date: 23 January 2020
Accepted Date: 03February 2020
Published Date: 05February 2020

***Corresponding author:**
Larissa M. Williams
Bates College
Department of Biology
44 Campus Avenue
Lewiston ME 04240 USA
E-mail: lwillia2@bates.edu

Citation: Williams LM Bowsher AM, Chrysovergi MA, Ambrose Jr. WG. Bloodworm (Glycera dibranchiata Ehlers, 1868) Populations in the Gulf of Maine Are Connected Through Gene Flow. Mar Sci Biol. 2020 Feb;1(1):121

warming faster than 99% of the rest of the ocean [6], may further endanger this species and has contributed to its overall vulnerability rank as "very high" by NOAA (NMFS 2015).

*G. dibranchiata*is a semelparous, carnivorous, marine polychaete [7,8]. We know very little about the development of G. dibranchiata, but it is thought that most adults mature, spawn, and die after 3 years, though some have been shown to reach 5 years of age [7,9]. Spawning in the northeast, where up to 10 million eggs per female are released, mostly occurs in shallow water during late afternoon high tides and only lasts a few daysaround mid-May [7,9]. The duration of the larval stage has never been studied in the field. In the lab, fertilized eggs develop into a trochophore larva between 14-20 hours post fertilization and survive up to 17 days but not beyond the trochophore stage [7,10]. The congeneric Glycera capitate Ørsted develops a benthic form in about 21 days [11]. Glycera spp. larvae are rarely sampled from the plankton [12] so we assume that their time in the plankton is short. If they have a short plankton phase and limited dispersal as adults, then populations should be relatively genetically isolated.

Our paucity of knowledge on the reproductive biology, harvest practices, and genetics of *G. dibranchiata*in Maine means we do not have sufficient information to manage the fishery [13,14]. The results of starch gel electrophoresis of four *G. dibranchiata* enzymes were used to hypothesize that a genetic bottleneck had occurred in one Maine population (Cod Cove) due to overharvesting [15]. Following up, Bristow and Vadas [16] used starch gel electrophoresis of 10 enzymes to assess the genetic variation of *G. dibranchiata*from eight intertidal sites in the Gulf of Maine and the Atlantic coast of Nova Scotia. They found intra- and inter-estuarine genetic differentiation indicating low gene flow [16],although much of this variation was driven by Cod Cove, the population that had recently collapsed. These studies were important first steps in understanding the population genetics of bloodworms in Maine. Starch gel electrophoresis of proteins, however, is not as sensitive as DNA sequencing and uses enzymes that may be non-neutral and could be influenced by natural selection.

Therefore, we used a more accurate, modern, and neutral approach to assess populationstructure and gene flow among populations along t 106 he coast of Maine. We used the cytochrome c oxidase I gene, a gene that has been used as a neutral genetic marker [17] to help measure the maternal gene flow among bloodworm populations in order to assess the genetic linkage of populations in Maine.

Materials and Methods

Site locations

We selected seven mud flats across the Gulf of Maine and collected 30 worms per site from January through June of 2017 and in June of 2018. Professional diggers harvested worms from the middle to low intertidal and held them in buckets until worms were frozen upon return to the worm dealer. From south to north, the sites were: (A) Upper Rich Cove--URC (43° 49' 43.85'' N 69° 54' 969'' W), (B) Brookings Bay--BB (43° 55' 33.0384'' N69° 44' 26.4696'' W), (C) Cod Cove--CC (44° 0' 3.906'' N69° 37' 56.874'' W), (D) Round Pond--RP (43° 56' 52.296'' N69° 27' 40.1724'' W), Raccoon Cove--RC (44° 28' 1.776'' N68° 17' 2.1624'' W), Long Ledge--LL (44° 31' 10.6212'' N 68° 9' 52.9092'' W), and Jonesport--JP (44° 31' 51.762'' N67° 36' 10.836'' W) (Figure 1). Specimens were transported frozen to the laboratory and preserved at -20°C until processing.

Genomic DNA isolation, extraction, and amplification

DNA isolation was performed using the Qiagen DNeasy Blood and Tissue Kit. DNA was then quantified using a NanoDrop Spectrophotometer and a polymerase chain reaction (PCR) was performed on the cytochrome c oxidase I gene using universal primers [18]. PCR reactions were carried using RedTaq (ThermoFisher) and the thermocycling profile consisted of 30 cycles of 2 min at 94°C, 15 s at 94°C, 30 sat 53°C, 1 min at 72°C and of a final extension f 129 or 7 minutes at 72°C. PCR products were electrophoresed on a 1.5% gel and visualized.

Purification of PCR products and sequencing

The successfully amplified products were purified with the Qiagen QIAquick PCR

Purification Kit: The purified PCR products were Sanger sequenced in both directions using an Applied Biosystems 3130xl DNA sequencer at the Mount Desert Island Biological Laboratory.

Data analysis

Consensus sequences for each individual were produced from the forward and reverse sequences using ClustalX 2.1 [19]. Using DnaSP v.5 [20], genetic diversity was analyzed within and between populations by determining haplotype diversity (Hd), nucleotide diversity (P_i), and pairwise distances between populations (F_{ST}).

Results

We sequenced 486 nucleotides in each individual, yielding 13 haplotypes (Figure 1). Of the 210 individuals analyzed, 197 (93.8%) had the same haplotype ("haplotype 1"). There were 12 other haplotypes found, but all except haplotype 12 were represented by a single individual in a single population. Haplotype 12 was shared by one individual in Long Ledge and one in Jonesport. Upper Rich Cove, Cod Cove, and Round Pond harbored only haplotype 1 and the most diversity existed in Long Ledge that had 10 haplotypes yet this site only had a small nucleotide diversity of 0.00246.

Genetic differentiation between two populations [21] which is measured by the fixation index, F_{ST}, was calculated. There was very little to no genetic differentiation between populations in the south (determined by an FST of less than 0.05; [22], and there was only moderate differentiation (determined by an F_{ST} between 155 0.05 and 0.15) between Long Ledge and the most southern site, Upper Rich Cove (Table 2).

Discussion

As a vital bait worm used in several fisheries in Maine and exported worldwide [13], and a species that is vulnerable to climate change NOAA [23], it is important to understand how G. dibranchiata populations are genetically connected. In their 1991 paper on *G. dibranchiata*genetics, Bristow andVadas [16] found moderate levels of genetic variation within populations, moderate levels of differentiation among populations, and thus low levels of genetic exchange among populations. The population that was contributing most to the variation they observed was Cod Cove, a population that had no COI genetic diversity in our study (Figure 1). At our neutral, maternally-derived, marker we show that there is limited population differentiation and low levels of variation within populations indicating gene flow along the section of Maine coast we sampled.

Long Ledge harbored the greatest genetic diversity with 10 haplotypes sequenced compared to 3 at the next most diverse site, Jones Port (Figure 1). Diggers harvest from many different intertidal mudflats and return to worm dealers to sort their catch, culling out 'shorts', worms that are below market size, in the process. Some diggers make an effort to return these shorts to a convenient site, which is rarely the site where they wereharvested. Diggers have been dumping their 'shorts' a 174 t Long Ledge for at least two generations (Harrington pers. comm.). Therefore, the high genetic diversity at this site is, easily explained and is the first evidence that returned worms survive at least long enough to be harvested by our diggers. The two most likely explanations for the discrepancy between our study and Bristow et al's [16] study are: 1) the enzyme markers used in 1991 were under selection since they are protein coding genes and are known to be non-neutral; and/or 2) we lose clarity of within and

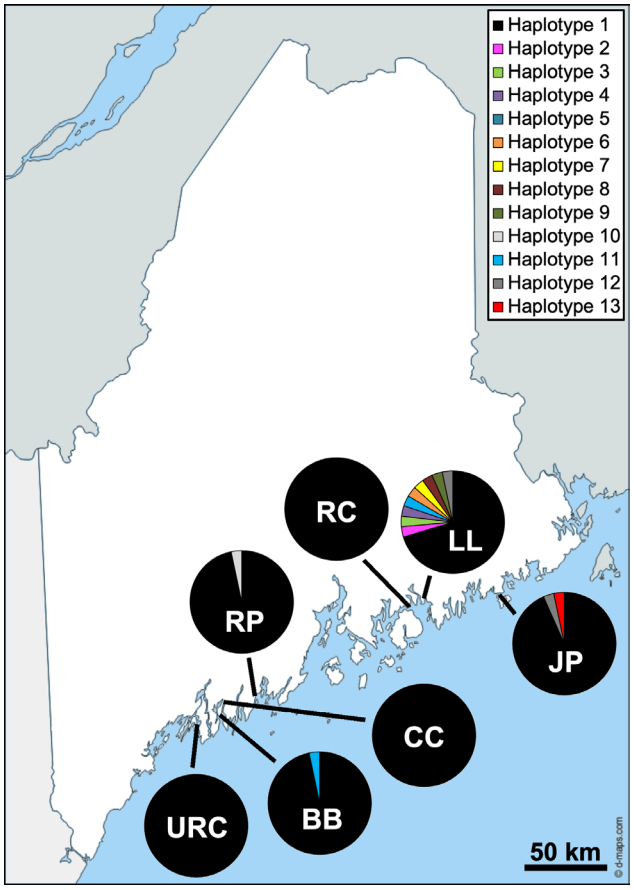


Figure 1: Cytochrome c oxidase subunit I haplotype frequencies for Glycera dibranchiate (n=30) at sampling locations in the Gulf of Maine. Pie charts indicate the proportion of haplotypes present at each site.

between population dynamics by only assessing a single maternally-derived gene with one allele. If the genes Bristow et al. [16] assayed were under selection (as they suggest in their discussion), it would indicate a greater amount of divergence between populations than a neutral maker like COI.

It is difficult to reconcile the lack of genetic diversity we found among populations and the reproductive behavior of *G. dibranchiata*. A larval dispersal stage of only a few weeks does not lend itself to wide dispersal and connections among estuaries. Adult worms enter the water column for reasons other than reproduction [22], but the frequency of this behavior and the extent to which it results in the dispersal of worms is unknown. Without more information on the larval development of *G. dibranchiata*in the Gulf of Maine and the behavior of post-settlement individuals, any explanations for the low genetic diversity we recorded would be speculative.

We can obtain better resolution on the extent of the differentiation if we sequence microsatellite markers. Like COI, microsatellites are neutral markers, but are part of the nuclear genome and thus have two alleles allowing for heterozygosity and Hardy Weinberg estimates that may better determine the within and between populationdynamics of *G. dibranchiata*[24]. These markers have not been developed for *G. dibranchiata*so primers for other polychaetes [25-27]could be tried. Nevertheless, our results suggest that the *G. dibranchiata*populations in the Gulf of Maine are well connected.

Our results have important implications for the management of bloodworms in the Gulf of Maine. Based on the results of Bristow and Vadas [16], fisheries managers in Maine have considered managing populations on an estuary scale. The lack of geneticdifferentiation we found among widely separated populations indicates, however, thatmanagement and conservation efforts should be applied at the scale of the Gulf of Maine.

Acknowledgments

This work would not have been possible without the help and insights of Peter Thayer of the Maine Department of Marine Resource and Dan Harrington. We thank Glen Clark, Dan Harrington, Fred Johnson, and John Renwick for harvesting worms and giving them special treatment. Thanks to Dr. Joshua Lord for the help in the design of Figure 1 and Isabelle Oliver for help in the design of the graphical abstract.

Funding

Research reported in this project was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103423 and funds from the Maine Department ofMarine Resources (Contract 13A 2018 0110*2159).

Data Availability

Haplotype sequences have been deposited into GenBank through accession numbers MN958890-MN958902.

References

1. Victoria C, Chick R, Hutchings P. A review of global fisheries for polychaete worms as a resource for recreational fishers: diversity, sustainability and research needs. Reviews in Fish Biology and Fisheries. 2018 Sep;28(3):543–565.
2. Gordon W, Murray J, Schaefer M, Bonner A. Bait worms: A valuable and important fishery with implications for fisheries and conservation management. Fish and Fisheries. 2016.
3. Dow RL, Jr. Creaser EP.Marine bait worms: a valuable inshore resource.Marine resources of the Atlantic coast. In Atlantic States Marine Fisheries Commission. Tallahassee, Florida.1970.
4. DMR, Maine. 2018.
5. Brown, B. Maine's Baitworm Fisheries: Resources at Risk? American Zoologist.1993 Dec;231 33(6):568-577.
6. Pershing AJ, Alexander MA, Hernandez CM, Kerr LA, Le Bris A, et al. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science. 2015 Nov 13;350(6262):809-812.
7. Klawe WL, Dickie LM. Biology of the bloodworm,Glycera dibranchiate Ehlers, and its relation to the bloodworm fishery of the Maritime Provinces. Bulletin of Fisheries Research Board of Canada. 1957;115:1-37.
8. Ambrose WG., Jr. Influences of predatory polychaetes and epibenthic predators on the structure of a soft bottom community in a Maine estuary. J Experimental Marine Biology Ecology, 1984 Oct;81(2):115-145.
9. Creaser EP., Jr. Reproduction of the bloodworm (*Glyceradibranchiata*) in theSheepscot Estuary. Journal of the Fisheries Research Board of Canada. 1973;30:161-166.
10. Simpson M. Gametogenesis and early development of the polychaete*Glyceradibranchiata*. The Biological Bulletin. 1962;123(2):412-423.
11. Lebskii, VK. Development of GlyceracapitataØrsted and Aonidespaucibranchiata Southern (Annelides, Polychaeta). Tr. BelomorBiol St MGU. 1970;259(3):91-97.
12. Omel'yanenko VA, Kulikova VA. Composition, Seasonal Dynamics, and Long-Term Fluctuations in the Density of Pelagic Polychaetes in Amurskii Bay, Sea of Japan. Russian Journal of Marine Biology, 2002 Sep;28(5):308-316.
13. Sypitkowski EWG. Ambrose Jr., Bohlen C, Warren J. Catch statistics in the bloodworm fishery in Maine. Fisheries

Research.2009;96(2-3):303-307.

14. Sypitkowski E, Ambrose Jr. WG, Bohlen C, Warren J. Harvest Efficiency of Bloodworms in Maine. North American Journal of Fisheries Management. 2008 Feb;28(2):284 1506-1514.
15. Vadas RL, Bristow GA. Genetic changes associed with a bottleneck in anoverharvested population of *Glyceradibranchiata* (polychaeta) (John Wiley andSons: New York). 1985;617-629.
16. Bristow GA, Vadas RL. Genetic variability in bloodworm (Glyceradibranchiata) populations in the Gulf of Maine', Marine Biology. 1991 June;109(2):311-319.
17. Joe R. Diluting the founder effect: cryptic invasions expand a marine invader's range. Proc Biol Sci. 2006 Oct;273:2453-2459.
18. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.Mol Mar BiolBiotechnol. 1994 Oct;3(5):294-299.
19. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA. Clustal W and Clustal X version 2.0. Bioinformatics. 2007 Sep;23:2947-2948.
20. Librado P, Rozas J. DnaSP v5: a software 260 for comprehensive analysis of DNA polymorphism data', Bioinformatics. 2009 Jun 1;25(11):1451-1452.
21. Nei M, Chesser RK. Estimation of fixation indices and gene diversities. Annals of human genetics. Ann Hum Genet. 1983 Jul;47(3):253-259.
22. Hartl DL. Principles of population genetics (Sinauer Associates: Sunderland, Massachusetts).1980.
23. NMFS. 2015.
24. Dean D. The swimming of bloodworms (*Glycera spp.*) at night, with commentson other species. Marine Biology. 1978;48(1):99-104.
25. Vieira MLC, Santini L, Lima Diniz A, de Freitas Munhoz C. Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology. 2016 Sep. 39(3):312-328.
26. Weinmayr G, D. VautrinM. Solignac. Isolation and Characterization of Highly Polymorphic Microsatellites from the PolychaetePectinariakoreni. Marine Biotechnology. 2000 Jan;2(1):92-99.
27. McMullin, ERJ. Hamm WS. Twelve microsatellites for two deep sea polychaete tubeworm species, *Lamellibrachialuymesi*and *Seepiophilajonesi*, from the Gulf of Mexico. Molecular Ecology Notes. 2003 Nov;4:1-4.