THE EFFECTS OF PELAGIC LARVAL DURATION ON THE GEOGRAPHIC POPULATION STRUCTURE OF TWO SIPUNCULAN SPECIES IN THE SEA OF JAPAN

A Senior Scholars Thesis

by

LAURA TIMM

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

May 2012

Major: Marine Biology

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Approved by:

Research Advisor: Anja Schulze
Associate Director, Honors and Undergraduate Research: Duncan MacKenzie

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ABSTRACT

The Effects of Pelagic Larval Duration on the Geographic Population Structure of Two Sipunculan Species in the Sea of Japan.
(May 2012)

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This study examines genetic diversity in two species of sipunculan worm, *Phascolosoma agassizii* and *Themiste pyroides*, in the Sea of Japan. Low sea levels of the Pleistocene era partially or completely isolated marginal seas of the northwestern Pacific, including the Sea of Japan. *Themiste pyroides* exhibits a larval stage of approximately 15 days, about half as long as the 31-day pelagic larval stage of *P. agassizii.* These differences in pelagic larval duration (PLD) may impact geographic population structure. I hypothesize that a longer PLD will result in increased inter-population gene flow and genetic homogeneity in *P. agassizii*. For both species, we sampled four populations within the Sea of Japan. We then compared the inter-population and intra-population genetic diversity. Analyzing sequence data from 16S ribosomal RNA (16S) and cytochrome *c* oxidase subunit I (COI) with the Analysis of Molecular Variance (AMOVA) statistic, we found substantially higher geographic population structure in *T. pyroides* than in *P. agassizii*. These results may support a direct correlation between PLD and gene flow,

indicating a dynamic relationship between life history traits and geographic population structure.

DEDICATION

This work is dedicated to the many teachers who have helped me on my way to a career in science: Ms. Bueller, for teaching me how to learn; Mr. Grundmann for introducing me to the rigors of science; Mr. Bentz, for reminding me, at a necessary time, to enjoy learning; Mr. Adams, for teaching me to write; Mr. Forseth, for challenging my presuppositions; Mr. Melendez, for illustrating that answered questions are intellectual hotbeds for inquiry; Ms. Luby, for showing me how to get here; and to Rebecca Timm for teaching me first, last, and longest.

ACKNOWLEDGMENTS

I would like to thank Dr. Anastasia Maiorova for collecting and shipping specimens from the Sea of Japan; Dr. Mary Rice for her insights into sipunculan life history traits; and members of the Schulze lab for unflagging support. The research was funded by a collaborative grant from the Far East Branch of the Russian Academy of Science and CRDF Global to AS and AM (RUB1-2996VL-11), by NSF AToL grant DEB-1036186 to AS. Special recognition is due to my thesis advisor, Dr. Anja Schulze for all of the guidance and advice she has provided over the course of the project.

NOMENCLATURE

PLD	Pelagic larval duration
COI	Cytochrome *c* oxidase subunit I
16S	16S ribosomal RNA subunit
PCR	Polymerase chain reaction
AMOVA	Analysis of molecular variance
h	Haplotype-diversity
π	Nucleotide-diversity
df	Degrees of freedom

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CHAPTER I

INTRODUCTION

Species are usually separated from each other by a certain amount of genetic divergence. This may also be the case for geographically isolated populations of the same species. The genetic differences between species or regional populations are caused by different environmental factors between the regions, either presently or historically, or by genetic drift. Intraspecific diversity within a region is usually a function of other factors, such as life history characteristics and the peculiarities of the sampling locations within the region. In many marine organisms, an important life history characteristic is the pelagic larval duration (PLD). A pelagic larval stage is seen in many marine taxa and serves as the dispersal phase in many species (Lalli and Parsons, 1997). In enclosed seas, which can have populations of several related species in environmentally similar though geographically separated locations, the length of the PLD may impact genetic mixing among these populations.

A marginal sea in the Western Pacific, the Sea of Japan has limited connections to the Pacific through the Strait of Tartary, the Le Perouse Strait, Tsugaru Strait, the Kanmon Strait, and the Korea Strait. However, the low sea levels of the Pleistocene partially or

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This thesis follows the style of Marine Biodiversity.

completely closed off these connections (Kitamura et al., 2001). The land barriers caused geographic isolation, leading to allopatry in many marine species, as has been studied and confirmed in several fish species (Higuchi and Goto, 1996; Liu et al., 2007), as well as three species of Sipuncula (Schulze et al., in press), two of which were the focus of this study.

Members of Sipuncula typically exhibit a pelagic larval phase of varying length, although some develop directly into the mature adult form (Adrianov and Maiorova, 2010; Rice, 1967). Differences in PLD may have significant impacts on geographic population structure. A longer PLD allows sipunculan larvae more time to relocate, possibly settling in a different population; whereas a shorter PLD does not promote larval dispersal far from the parent population. PLDs potentially have strong impacts on the magnitude of gene flow among populations.

The two species of Sipuncula analyzed in this study are distinct from each other. Schulze et al. (in press) have shown that even though both species are reported as cosmopolitan (Cutler, 1994), they represent complexes of cryptic species throughout their distribution range. Cryptic species are species that are morphologically indistinguishable, yet constitute separate, genetically distinct species. In sipunculans, populations of each species in the Sea of Japan and the North American west coast are morphologically identical; however, Adrianov and Maiorova (2010) and Rice (1967) have found the species differ in reproductive and developmental timing, gametogenesis and

development rate. The two species analyzed in this study, *Phascolosoma agassizii* and *Themiste pyroides* exhibit different PLDs. *P. agassizii* has a larval stage that lasts approximately 31 days in the Sea of Japan (Adrianov and Maiorova, 2010). *T. pyroides* has a larval stage lasting about half that long, averaging about 15 days.

Mitochondrial DNA has a high rate of mutation, which is very helpful in elucidating geographic population structure in a species. Two mitochondrial gene regions have been sequenced: cytochrome *c* oxidase subunit I (COI) and 16S ribosomal RNA (16S). These markers are frequently used in phylogeographic and population genetics studies of invertebrates (e.g. Bastrop et al., 1998; Jolly et al., 2004; Schulze, 2006; Kawauchi and Giribet, 2010). COI generally varied enough to distinguish species and often populations (Folmer et al., 1994). 16S is a conservative macromolecule that has become widely recognized and used to determine phylogenetic placement of bacterial species (Woese, 1987). It is also frequently utilized to elucidate phylogenetic analyses of invertebrate taxa (Schulze, 2006).

This study focuses on analyzing the genetic diversity found in populations of two species of sipunculan worms from several populations within the Sea of Japan. Considered a phylum, recent molecular data and developmental studies suggest Sipuncula is closely related to, if not part of the Phylum Annelida (e.g. Kristof et al., 2008; Struck et al., 2007). These unsegmented worms are good model systems for phylogeographic and

population genetic studies because they are common and have a wide geographic distribution.

This study aims to analyze genetic diversity within and among populations of *P. agassizii* and *T. pyroides* in the Sea of Japan as a function of their respective PLDs. I hypothesize that PLD will not show a strong effect on intra-population genetic variation. As the degree of gene flow between populations may be largely determined by the specific PLD, I further hypothesize that *T. pyroides* will show greater inter-population genetic divergence than *P. agassizii*. This difference would indicate higher rates of gene flow in *P. agassizii*, which exhibits a longer PLD.

CHAPTER II

MATERIALS AND METHODS

Themiste pyroides (Table 1) and *Phascolosoma agassizii* (Table 2) were collected from four locations in the Sea of Japan: Aleut Bay, Amursky Bay, Ussuriisky Bay, and Vostok Bay (Figure 1). Sequences were generated for *T. pyroides* (*n* = 40) and *P. agassizii* (*n* = 54), aligned, and used to calculate the inter- and intra-population diversity of each species.

Collections

Specimens of *Themiste pyroides* and *Phascolosoma agassizii* were collected from Peter the Great Bay in the Sea of Japan by divers from the Institute of Marine Biology in Vladivostok and shipped to Texas A&M University at Galveston. They were collected from a variety of habitats: silted sand, seagrass rhizomes, and clusters of the mussel *Crenomytilus grayanus*. They were preserved in 95% ethanol.

In total, 52 specimens were collected and formed the basis of the geographic population structure study. *Themiste pyroides* (Table 1) and *Phascolosoma agassizii* (Table 2) were collected from four locations within the Sea of Japan.

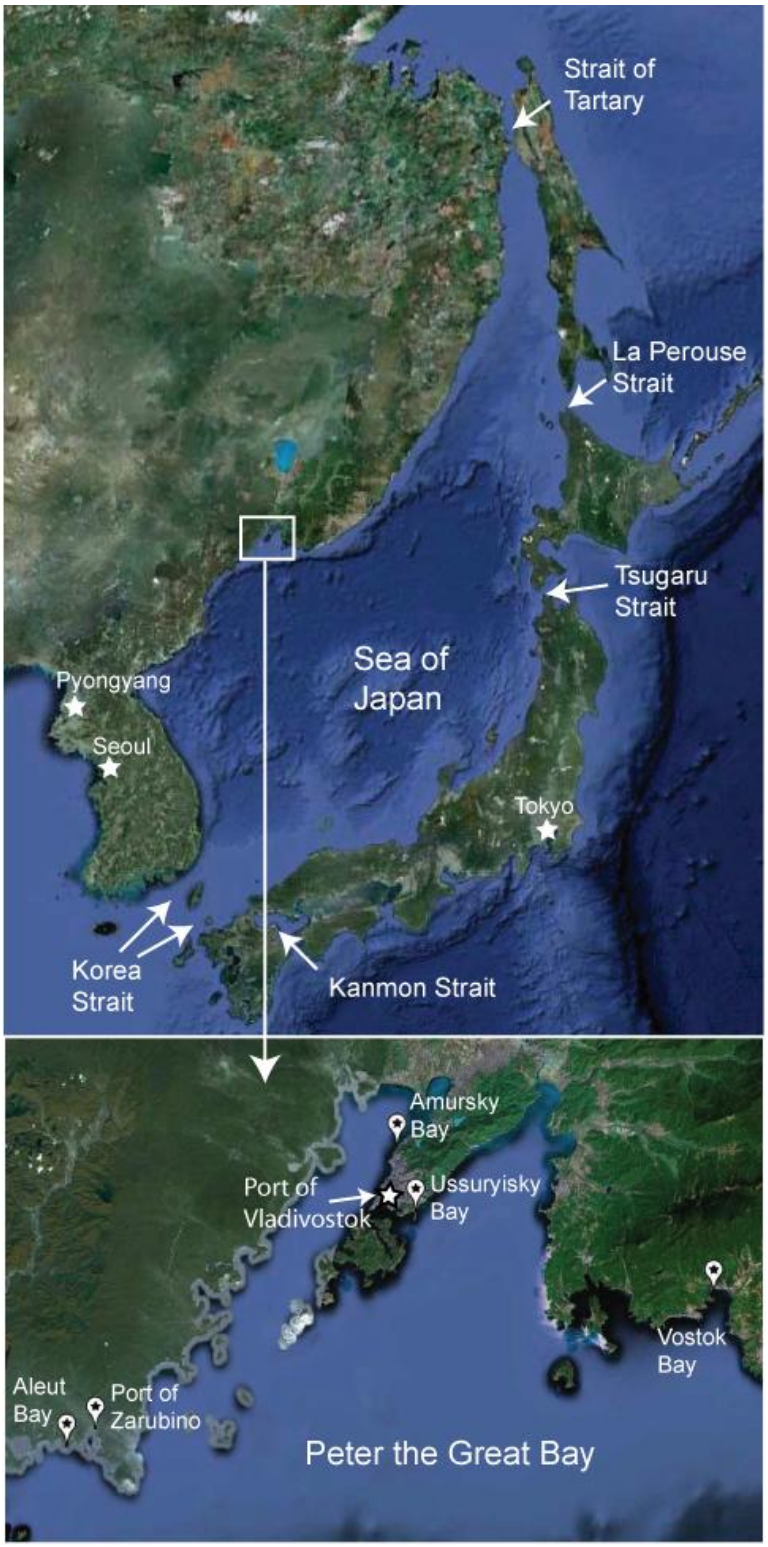

Figure 1. Map illustrating sample locations within the Sea of Japan. Top map is an overview of the region. Bottom map is a close-up of the collecting locations

Table 1. Data for the *T. pyroides* specimens used in this study. Each 16S and COI haplotype has been numbered. The 16S haplotype and the COI haplotype sequenced from each specimen are denoted in the *16S haplotype* and the *COI haplotype* columns.

Accession number	*Collection Location*	*Latitude and longitude*	*Collection date*	*16S haplotype*	*COI haplotype*
100329	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	1	1
100331	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	1	2
100332	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	1	3
100334	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	2	---
100351	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	1	3
100354	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	1
100355	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	4	3
100356	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100357	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100358	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	5	12
100359	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100360	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100361	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100362	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	---
100363	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	1	3
100395	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	1	3
100396	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	1	3
100397	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	8	19
100398	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	1	20
100399	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	9	21
100400	Vostok Bay	42°53'35.78"N 132°44'5.18"E	6/10/2011	1	3

Table 2. Data for the *P. agassizii* specimens used in this study. Each 16S and COI haplotype has been numbered. The 16S haplotype and the COI haplotype sequenced from each specimen are denoted in the *16S haplotype* and the *COI haplotype* columns.

Accession number	*Collection Location*	*Latitude and longitude*	*Collection date*	*16S haplotype*	*COI haplotype*
100339	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	4
100340	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	5
100341	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	6
100342	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	7
100343	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	8
100344	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	7
100345	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	---	9
100346	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	10
100347	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	---	11
100348	Aleut Bay	42°38'8.72"N 131°2'36.43"E	6/20/2011	3	7
100375	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	3	13
100376	Amursky Bay	43°11'55.74"N 131°55'10.09"E	6/16/2011	3	7
100377	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	6	7
100378	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	7	10
100379	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	3	14
100380	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	3	15
100381	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	3	10
100382	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	---	16
100383	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	---	7
100384	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	---	10
100385	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	---	17
100386	Ussuriisky Bay	43°4'24.73"N 131°57'51.36"E	6/30/2011	---	18
100401	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	7
100402	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	10
100403	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	22
100404	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	23
100405	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	10	24
100406	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	11	7
100407	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	7
100408	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	3	25
100409	Vostok Bay	42°53'30.06"N 132°44'5.88"E	6/10/2011	---	7

Sequence generation

Total genomic DNA was extracted using a DNeasy kit (Qiagen). Gene regions of the mitochondrial genes for large subunit ribosomal RNA (16S rRNA) and for cytochrome c oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR). PCR reactions were performed in a volume of 25µl. Each 25µl reaction consisted of 1µl of the extraction, 0.5 – 1 unit of taq polymerase (Lucigen), 2µM of dNTP (consisting of dATP, dGTP, dCTP, and dTTC), 1.25µM of each primer, and 2.5µM 1X PCR buffer were used for each reaction. PCR was performed using standard protocols with an annealing temperature of 45^{o}C for COI amplification and 40^{o}C for 16S amplification. The following primers were employed: 16Sar (5'-CGCCTGTTTATCAAAAACAT-3' [Palumbi, 1996]) and 16Sbr (5'-CCGGTCTGAACTCACATCACGT-3' [Palumbi, 1996]) for 16S rRNA; the primer pairs LCO (5'-GGTCAACAAATCATAAAGATATTGG-3' [Folmer et al., 1994]) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3' [Folmer et al., 1994]) were used for COI. PCR products were visualized using 1% agarose gels stained in ethidium bromide. 5µl of each PCR product were cleaned with 2µl Exo-Sap-IT (usb).

Sequence reactions were performed in10µl volume, using 2µl of the PCR product, 1µM of primer, 2µl of ABI BigDye Terminator ver. 3.1 (Applied Biosystems), 2µl 5X sequencing buffer (Applied Biosystems), and 3µl of milliQ water. Sequence reactions were performed in the forward and reverse direction with the same thermal cycler as for PCR reactions, using standard protocols. The sequence reactions were then cleaned up

using PrepEase Dye Clean-Up Columns (usb) and analyzed with an ABI 3130 genetic analyzer in the Marine Genomics Lab at Texas A&M University at Galveston. Electrochromatograms from the sequencer were visualized in Sequencher 4.8 (Gene Codes). Fragments of the forward and reverse reactions were assembled into contigs. Ambiguities in the contigs were reviewed and edited manually. Primer sequences were discarded. The sequences were aligned in ClustalW, as implemented in MEGA 5 (Kumar et al., 2009; Tamura et al., 2007).

Genetic diversity

Haplotype frequencies, haplotype-diversity, and nucleotide-diversity were calculated with Arlequin 3.5 (Excoffier et al., 2005). Finally, 16S and COI sequence data was used to perform Analysis of Molecular Variance (AMOVA) statistics. For AMOVA analysis, the four populations were placed in one group. Standard AMOVA computations were selected in haplotypic format with 1000 permutations. A distance matrix was computed using pairwise differences. The gamma a value was set to 0. AMOVA analysis yielded inter-population and intra-population genetic variance values for both species.

CHAPTER III

RESULTS

Intra-population diversity was calculated for *T. pyroides* using 16S (Table 3) and COI data (Table 4) and for *P. agassizii* using 16S (Table 5) and COI data (Table 6). The number of haplotypes per number of individuals within a population is an index of intra-population diversity. Of the 36 haplotypes used in this study, 25 were COI sequences and 11 were 16S sequences (Table 7). Within the 25 COI sequences, 18 of these haplotypes occurred in *P. agassizii* and 7 occurred in *T. pyroides*. The 11 16S haplotypes were divided more evenly, with 5 occurring in *P. agassizii* and 6 occurring in *T. pyroides.* Averaging the values from each population across species and genes, Vostok Bay had the highest haplotype number, followed by Aleut Bay and Ussuriisky Bay, with Amursky Bay showing the lowest average haplotype number. Ussuriisky Bay shows the highest average haplotype-diversity across species and genes, followed in descending order by Aleut Bay, Vostok Bay, and Amursky Bay. Nucleotide-diversity was highest in Vostok Bay, followed by Aleut Bay, Ussuriisky Bay, and Amursky Bay.

Table 3. Haplotype-diversity (h) and nucleotide-diversity (π) of *T. pyroides* 16S sequences

	Aleut Bay	***Amursky Bay***	***Ussuriisky Bay***	***Vostok Bay***
Number of individuals	4	10	1	6
Number of haplotypes	2	3	1	3
h	0.5000±0.2652	0.3778±0.1813	1.0000	0.6000±0.2152
π	0.0975±0.0645	0	0	0.0014±0.0014

Table 4. Haplotype-diversity (h) and nucleotide-diversity (π) of *T. pyroides* COI sequences

	Aleut Bay	***Amursky Bay***	***Ussuriisky Bay***	***Vostok Bay***
Number of individuals	3	9	1	6
Number of haplotypes	3	3	1	4
h	1.0000±0.2722	0.4167±0.1907	1.0000	0.8000±0.1721
π	0	0.0004±0.0005	0	0.3742±0.2167

Table 5. Haplotype-diversity (h) and nucleotide-diversity (π) of *P. agassizii* 16S sequences

	Aleut Bay	***Amursky Bay***	***Ussuriisky Bay***	***Vostok Bay***
Number of individuals	8	2	5	8
Number of haplotypes	1	1	3	3
h	0	0	0.7000±0.2184	0.4643±0.2000
π	0	0	0.0016±0.0016	0.0015±0.0014

Table 6. Haplotype-diversity (h) and nucleotide-diversity (π) of *P. agassizii* COI sequences

	Aleut Bay	***Amursky Bay***	***Ussuriisky Bay***	***Vostok Bay***
Number of individuals	10	2	10	9
Number of haplotypes	8	2	7	6
h	0.9333±0.0773	1.0000±0.5000	0.9111±0.0773	0.8333±0.1265
π	0.0064±0.0039	0.0015±0.0021	0.0290±0.0159	0.0048±0.0031

Table 7. Occurrence of each 16S and COI haplotype in each location. Bold face type means that the haplotype occurs in at least 3 locations

Haplotype	***Aleut Bay***	***Amursky Bay***	***Ussuriisky Bay***	***Vostok Bay***
16S (*T. pyroides*)	**1**, 2	**1**, 4, 5	**1**	**1**, 8, 9
16S (*P. agassizii*)	**3**	**3**	**3**, 6, 7	**3**, 10, 11
COI (*T. pyroides*)	1, 2, **3**	1, **3**, 12	**3**	**3**, 19, 20, 21
COI (*P. agassizii*)	4, 5, 6, **7**, 8, 9, 10, 11	**7**, 13	**7**, **10**, 14, 15, 16, 17, 18	**7**, **10**, 22, 23, 24, 25

The AMOVA statistic was run to compare inter-population genetic variability. AMOVAs were run with 16S data and COI data separately for each species. The AMOVA run using COI data from *T. pyroides* (Table 9) yielded the highest percentage of among-populations variance (= 36.03) while the 16S *P. agassizii* (Table 10) AMOVA yielded the lowest (= -4.44). The among-populations percent variance values falling intermediately reflected the same results, with the 16S *T. pyroides* AMOVA (Table 8) yielding a higher value than the COI *P. agassizii* AMOVA (Table 11).

AMOVA analysis also yielded percentages of intra-population variation (Tables 8-11). In both genes, *T. pyroides* showed a lower percentage of genetic variation due to intra-population variance compared to *P. agassizii*, however, the difference is relatively small.

Table 8. Results from 16S *T. pyroides* AMOVA analysis.

Source of Variation	*d.f.*	*Sum of Squares*	*Variance Components*	***Percentage of Variation***
Among Populations	3	19.036	0.44975 Va	9.49
Within Populations	17	72.917	4.28922 Vb	90.51
Total	20	91.952	4.73897	

Table 9. Results from COI *T. pyroides* AMOVA analysis.

Source of Variation	*d.f.*	*Sum of Squares*	*Variance Components*	***Percentage of Variation***
Among Populations	3	389.085	22.05356 Va	36.03
Within Populations	15	587.389	39.15926 Vb	63.97
Total	18	976.474	61.21282	

Table 10. Results from 16S *P. agassizii* AMOVA analysis.

Source of Variation	*d.f.*	*Sum of Squares*	*Variance Components*	***Percentage of Variation***
Among Populations	3	0.514	-0.00946 Va	-4.44
Within Populations	19	4.225	0.22237 Vb	104.44
Total	22	4.739	0.21291	

Table 11. Results from COI *P. agassizii* AMOVA analysis.

Source of Variation	*d.f.*	*Sum of Squares*	*Variance Components*	***Percentage of Variation***
Among Populations	3	16.204	0.13845 Va	3.05
Within Populations	27	118.667	4.39506 Vb	96.95
Total	30	134.871	4.53351	

CHAPTER IV

CONCLUSIONS

Data from this study shows that the vast majority of genetic variance in both species is from intra-population diversity. Thus gene flow is clearly occurring in both species. However, inter-population gene flow, possibly due to PLD, was seen to vary slightly between species.

Intra-population variation was gauged by haplotype number, haplotype- and nucleotide-diversity. In both species, COI typically generated a higher number of haplotypes than 16S. Moreover, a higher amount of haplotype-diversity and nucleotide-diversity was seen within COI compared to 16S. This is not surprising, as the COI gene is more variable than 16S. In *T. pyroides,* the number of haplotypes per number of individuals was 0.4286 for 16S and 0.3478 in COI. For *P. agassizii*, it was 0.5789 for 16S and 0.7419 for COI. The ratio between number of haplotypes and number of individuals across all populations was slightly greater in *P. agassizii* than in *T. pyroides*. However, the *T. pyroides* sequences exhibited higher haplotype- and nucleotide-diversity than *P. agassizii* in the individual populations (Tables 3-6). As both species exhibit relatively equal numbers of haplotypes, it seems that PLD does not impact intra-population genetic variation. Intra-population diversity seems to vary with location. The number of haplotypes and the nucleotide-diversity value rank the locations in descending order as follows: Vostok Bay, Aleut Bay, Ussuriisky Bay, and Amursky Bay. The haplotype-

diversity calculations, however, do not support the order. Ussuriisky Bay shows the highest haplotype-diversity across species and genes, but this location also had the fewest samples (n = 17). Aleut Bay (n = 25), Vostok Bay (n = 29), and Amursky Bay (n = 23), fell out in the same order as indicated by average haplotype number.

This trend may be explained by the degree of bay enclosure. Vostok Bay is located in a very enclosed inlet. Amursky Bay and Ussuriisky Bay are located on opposite sides of a peninsula, with Amursky Bay situated closer to the joining of the peninsula to the mainland. Aleut Bay is located on a slightly concave coastline. The more enclosed a bay is, the greater potential it has to keep sipunculans within its confines.

The effect of PLD on inter-population genetic divergence is much stronger than on intra-population diversity. The AMOVA results indicate a direct relationship between PLD and among-population genetic diversity: In both the 16S and the COI AMOVA analysis, *T. pyroides* exhibited a higher percentage of among-population variation when compared to the *P. agassizii* AMOVA values. These higher values indicate less genetic homogeneity among the four populations of *T. pyroides* when compared to the populations of *P. agassizii*. As *P. agassizii* has a PLD lasting around 31 days, larvae have more time in the water column to move from one population to another. *Themiste pyroides* has a PLD about half the length of *P. agassizii*, greatly abbreviating the time spent in the water column before settling into a semi-sedentary lifestyle. This difference

in time spent in the dispersal phase could impact gene flow between populations, contributing to the differences seen in the percentage of among-population variation.

Ports may also serve as vectors of gene flow. A small port, the Port of Zarubino, is located near Aleut Bay and the large Port of Vladivostok is located in between Amursky Bay and Ussuriisky Bay. The ship traffic from these areas may well impact diversity on both the intra- and inter-populations levels. The presence of the Vladivostok Port may explain the high intra-population variability in that area. However, the Port of Zarubino does not seem to have affected Aleut Bay similarly. These ports may impact inter-population diversity by transporting larvae in ballast water. Thus, while PLD seems to have some impact on intra- and inter-population diversity, it is clear that other factors impact genetic homogeneity within the Sea of Japan.

The results of this study support my hypotheses: *T. pyroides*, a sipunculan species with a shorter PLD, exhibited a high percentage of among-population variance compared to *P. agassizii*, and both species had similar percentages of within-population variance. The high percentage of intra-population genetic variation confirms gene flow in both species, however differences in inter-population variation between species indicate the potential impact of PLD to alter genetic population structure even on a small geographic scale.

The impacts of pelagic larval duration on survivorship in marine fishes are fairly well understood (Leggett and Deblois, 1994; Suthers, 1998; Bergenius et al., 2002), but its

role in gene flow in marine invertebrates has not yet been fully determined. In the future, I would like to investigate regional differences between *P. agassizii* and *T. pyroides* in the Sea of Japan and the Northeast Pacific. According to Schulze et al. (in press), PLD of these species differs between regions. Studying geographic population structure between species between regions could better elucidate the relationship between PLD and gene flow within and among populations. These results may support a direct correlation between PLD and gene flow, indicating a dynamic relationship between life history traits and geographic population structure.

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