Ecology and taxonomy of the early life stages of arrowtooth flounder (Atheresthes stomias) and Kamchatka flounder (A. evermanni) in the eastern Bering Sea

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Introduction
Arrowtooth flounder occur from central California, north to the Bering Sea, and west to the Kamchatka Peninsula, Russia. In the Bering Sea they feed primarily on juvenile and adult walleye pollock, euphausiids, and various shrimps. An increase in population size and recent interest for increased directed fishing effort has created a need to fully understand arrowtooth flounder in all life stages for management and ecological purposes. However, this has been confounded by the presence of Kamchatka flounder, a species which occurs mostly in the western Bering Sea, but can also be found in the eastern Bering Sea and along the Aleutian Islands. Identification between these two species is possible in the adult stage, but not in larval or early juvenile stages. The purpose of our study was 1) to use a genetic technique to identify larval and early juvenile Atheresthes spp. individuals collected in the eastern Bering Sea to the species level, 2) use genetically identified individuals to develop a visual identification method that would allow identification of historic and future samples, and 3) describe the ecology of the early life stages of both arrowtooth flounder and Kamchatka flounder by examining distribution, abundance, and bioenergetics.

Genetic Identification
A total of 415 Atheresthes spp. specimens were used for genetic identification. The size range of specimens was 6.0–300 mm standard length (SL), with the majority of specimens 6.0–115 mm SL. A single eyeball was used for DNA extraction using a standard Chemagic DNA extraction protocol. A 750 bp segment of cytochrome oxidase subunit I (COI) was amplified using PCR. A restriction enzyme digest using Bmt1 was used to cut the PCR product at a unique nucleotide sequence present only in arrowtooth flounder. The DNA fragments were visualized using gel electrophoresis.

Identification was based on either the presence of a single 750 bp fragment (Kamchatka flounder) or two fragments consisting of 450 and 300 bp (arrowtooth flounder).

Results
• Successful identification of specimens 6.0–120 mm SL and ≥18.0 mm SL
• Specimens 12.3–17.9 mm SL still indistinguishable due to high degree of natural variation and lack of sufficient specimens to examine.

Distribution
A total of 2,524 formalin-preserved Atheresthes spp. larval specimens collected on AFSC cruises conducted from 1994 to 2010 were selected for identification to the species level using visual identification. All specimens were categorized as either larvae (3.0–24.9 mm SL) or juveniles (25.0 mm SL).

Results
• Identifications from preserved specimens:
  • 1,547 arrowtooth flounder larvae and 125 juveniles
  • 480 Kamchatka flounder larvae and 8 juveniles

Visual Identification
All specimens used in the genetic technique were examined with regards to morphology, pigmentation patterns, and intensity of pigmentation. Additionally, 20 arrowtooth flounder and 19 Kamchatka flounder larvae, chosen from a single cruise, were measured for morphometric analysis. These specimens ranged in size from 6.3 to 12.6 mm SL.

Distribution
• Larvae and early juveniles identified to species in the laboratory and at sea:
  • 165 arrowtooth flounder
  • 194 Kamchatka flounder

Distribution of genetically identified specimens.
• Genetic results confirm both species are collected in the eastern Bering Sea, at similar locations and often in the same tow.

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Bioenergetics
Relative conditions and nutritional quality of specimens used was determined by examining energy density and lipid composition. Energy density (kJ/g dry mass) was estimated using bomb calorimetry after dried tissue was homogenized. Lipid composition was determined using a sufto-phospho-vanillin (SPV) colorimetric analysis and is presented as a percentage of the individual dry weight (% lipid). Stomach contents of fish were removed prior to chemical analysis so as not to affect estimates of energy density or % lipid.

Results
• No significant difference in energy density was observed between species, however:
  • Kamchatka flounder had slightly higher energy values regardless of life history stage.

• Larval Kamchatka flounder had higher lipid content than larval arrowtooth.
  • Larval Kamchatka flounder used in this study were larger than larval arrowtooth flounder (p<0.0001).
  • By juvenile stage, lipid content was similar between the species.

Distribution and abundance of visually identified arrowtooth flounder specimens.
• Larval arrowtooth flounder and Kamchatka flounder have very similar distributions in the eastern Bering Sea with the majority collected between Unimak and Unimak Pass and south of the Pribilof Islands.
• Juveniles of both species collected mostly north of where the majority of larvae occur.
  • Mean distribution of arrowtooth flounder juveniles is located farther east and over shallower waters than Kamchatka flounder juveniles. However, there were far fewer Kamchatka flounder juveniles collected than arrowtooth flounder juveniles.

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Conclusions
This study has provided successful methods for genetically identifying larval and early juvenile arrowtooth and Kamchatka flounder, and for visually identifying these two species at small (6.0–12.0 mm SL) and large sizes (≥18.0 mm SL). Focused field work in the future is necessary to sample in the appropriate areas and times to collect Atheresthes spp. larvae 12.1–17.9 mm SL to aid in determining diagnostic morphological and pigmentation characters so that specimens in this size range can be visually identified. Our study demonstrates that while the larvae and early juveniles of these two species exist in the same geographic areas during the same time, the bioenergetics analyses indicate potential niche partitioning in the larval stages between these two species. As arrowtooth flounder and Kamchatka flounder are potential sibling species that occur in the same geographic location and in the same temporal frame, it is important to understand how these two species interact with each other and how they may differ in their small-scale distribution (horizontal and vertical) and nutritional value (bioenergetics) to successfully co-occur and recruit to the adult life stage.

Acknowledgements
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