

Project No: F0522

Title: Reproductive ecology of Atka mackerel, *Pleurogrammus monopterygius*, in Alaska

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Contract Period and Amount of Funding: 5/01/2005 to 4/30/2007 for \$499,630 (extended to 12/31/2007)

Report Period: 01/01/2007 to 06/30/07

Report Date July 15, 2007

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**Project Summary:** Atka mackerel support a multi-million dollar commercial fishery and play a key role in the marine ecosystem of the Aleutian Islands. This study represents an ongoing research effort examining aspects of Atka mackerel reproductive ecology which will be directly applicable to estimates of spawning biomass, recruitment, stock dynamics, and distribution patterns. In 2004, spatio-temporal patterns in distribution were examined with respect to spawning condition and habitat use for nesting sites. Additionally, Atka mackerel embryos were incubated at different temperatures at the Alaska Sea Life Center to allow the construction of developmental series. This information is essential since egg development at low temperatures in deeper waters could extend the spawning season dramatically and influence distribution patterns. Variability in female maturity schedule was examined and it is proposed to estimate variability of realized fecundity that also directly affects reproductive output and estimation of female spawning biomass. Male spawning biomass might influence reproductive success as much as female spawning biomass due to nest guarding. Egg cannibalism as a mating strategy could maximize individual reproductive success and minimize the energetic costs associated with nest tending in males.

**Specific objectives of this study are to:** 1) analyze additional years of spatio-temporal distributions by reproductive stage, 2) expand embryonic developmental series over finer sampling scales and broader temperature range; 3) determine parentage of egg batches 4) evaluate egg cannibalism using genetic techniques; 5) investigate spatio-temporal variation in reproductive output (maturity schedule, realized fecundity); 6) determine fecundity and egg caloric content of successive batches spawned in captivity

**Progress summary:** Following is summary of progress made for the specific objectives

1) Analyze additional years of spatio-temporal distributions by reproductive stage.

Histology has been completed on samples collected aboard the F/V Seafisher during spawning season in 2005. A manuscript of the results of this objective is in preparation.

2) Expand embryonic developmental series over finer sampling scales and broader temperature range.

The incubation temperatures for 2006 had little variation (Figure 1). A total of 35 incubation experiments have been conducted from 2004 through 2006 at 11 temperature regimes (Table 1). Preliminary results indicate that eggs incubated below the published lethal temperature of 3°C at 1.6°C developed and survived through hatching. Eggs incubated above temperatures observed *in situ* did develop faster. However, embryos survived through hatching at 12.18°C and died before hatching at 14.98°C (Figure 2).

Hallmarks for morphological descriptions have been established (Table 1) and staging of eggs from the 2005 and 2006 season are on going. Preliminary staging of eggs from fertilization to complete epiboly has indicated that subsample frequency was adequate to document each stage of early development at all temperatures.

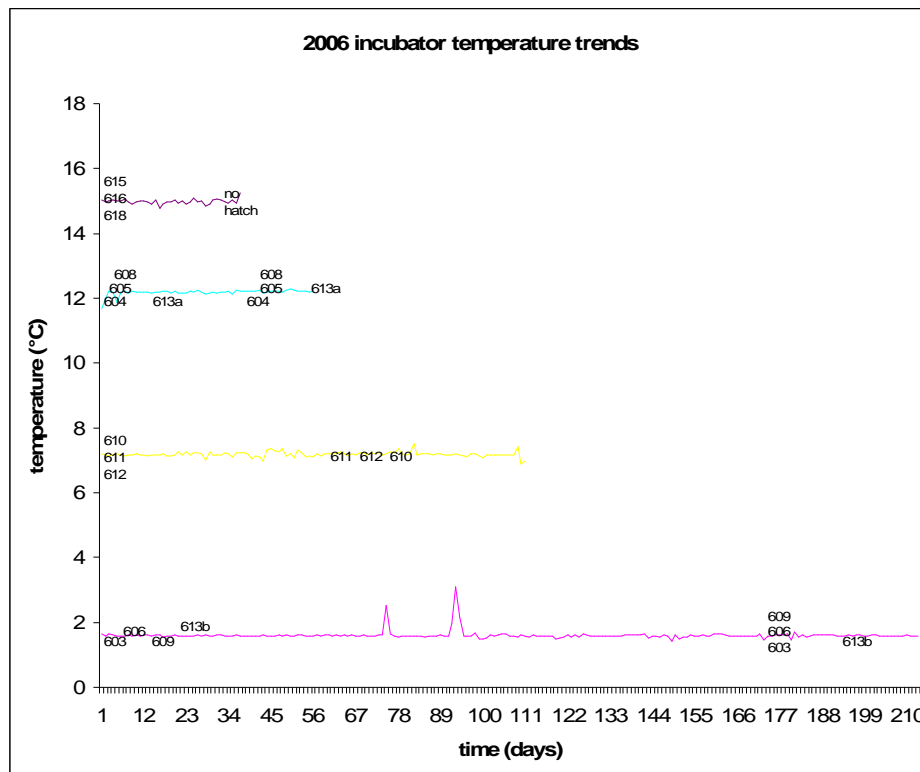


Figure 1. Graph showing egg mass temperature trends for each regime in 2006. Mean temperatures were 1.6°C, 7.19°C, 12.18°C and 14.98°C. Paired numbers indicate the

duration of incubation for an individual egg mass with the first number representing fertilization and the second representing hatch.

Table 1. Summary of temperature regimes for 2004 through 2006 with corresponding mean time until first hatch and standard error.

Study Year	Temperature Regime	Mean time until first hatch	Std. Error	n
2006	1.6	168.8	3.3	4
2004	3.89	100.7	0.333	3
2005	3.92	96.6	0.559	3
2004	5.03	84	0.577	3
2005	6.66	63.7	0.523	3
2004	7.02	64.7	1.201	3
2006	7.19	67.5	4.493	3
2005	9.85	40.9	0.61	4
2004	9.89	43.5	1.5	2
2006	12.18	39.1	0.883	4
2006	14.98	No hatch	-	3

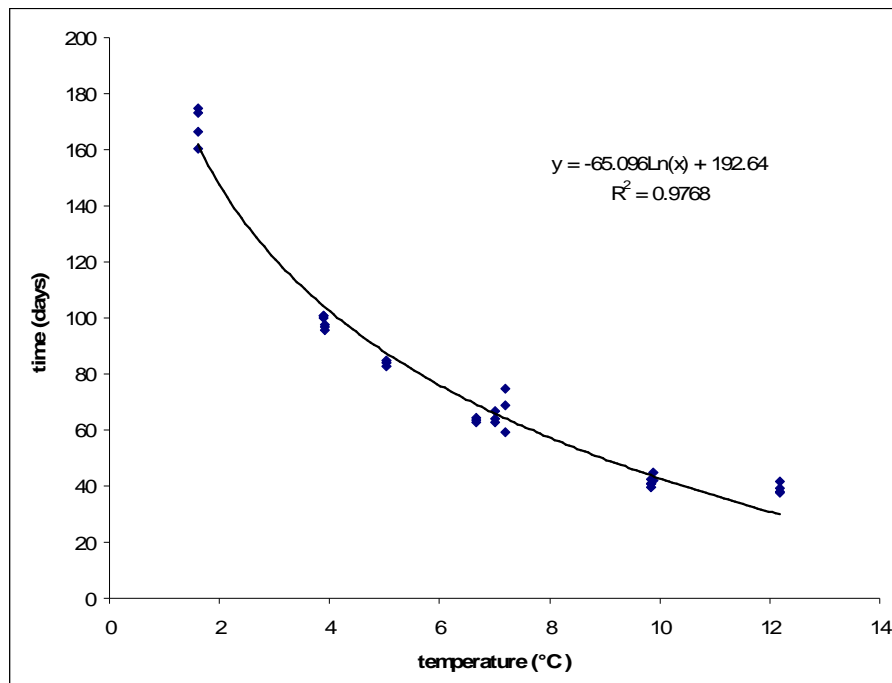


Figure 2. Time until first hatch for each temperature regime.

Table 2. Descriptions of hallmarks that will be used to stage the development of *Pleurogrammus monoptyerius*.

Stage	Description and hallmarks
Zygote	Fertilization, cytoplasm migration to the animal pole
Cleavage	First cleavage, Two-cell Second cleavage, Four-cell Third cleavage parallel to the second, Eight-cell Fourth cleavage parallel to the first, 16-cell Fifth cleavage, 32-cell Sixth cleavage, 64-cell
Blastula	blastodisc blastodermal cap Formation of germ ring and embryonic shield
Gastrula	1/4 epiboly 1/2 epiboly 3/4 epiboly Blastopore
Neurula	Formation of neural groove Neural keel visible Kuppfer's vesicle formation Blastopore closure Cranial regionalization, optic vesicles form
Embryo	Somites visible Tailbud or tail lift pigmentation on body Appearance of pectoral fins pigmentation of the eye Embryo surrounds the yolk 180 Embryo surrounds the yolk 270 Embryo surrounds the yolk 360
Hatching	First hatch 50% hatch 100% hatch

### 3) *Determine parentage of egg batches.*

Parentage was assessed for embryos in egg masses produced in captivity under two experimental conditions: a **small tank** (~ 10,200 l, 1.5 m depth) used for determining fecundity and egg energy content of individual females and a **large tank** (~ 400,000 l, 6.4 m in depth) exhibit at the Alaska SeaLife Center. Adult Atka mackerel had been successfully spawning in the large tank for several years and we expected that it would provide a more 'natural' habitat, mimicking spawning conditions in the wild. Lengths, weights, and fin clips were collected for all Atka mackerel used in both experiments. Natural markings from 6 known males in the large exhibit tank were documented for subsequent visual identification of males guarding territories.

#### **Small tank results:**

##### **2004 spawning**

Thirteen egg masses, produced by two males and four females in captivity at the Alaska SeaLife Center in 2004, were genotyped using DNA microsatellite markers to determine parentage. We genotyped 96 eggs per clutch for the first two clutches and determined that a single pair of parents was responsible for each clutch and that a single male dominated spawning events. These results were not consistent with those observed for egg masses collected in the field, where multiple parentage is very common, and we attribute them to the controlled conditions for mating in captivity. We elected not to exhaustively sample the remaining egg masses and instead analyzed 24 embryos from each. Twelve of the 13 egg masses appeared to be produced by a single set of parents. The exception was a single egg mass that was primarily fertilized by Male 1, but had a small proportion (~10%) of eggs fertilized by Male 2. There was also some variation in the realized fecundity of the females; one female produced six of the 13 egg masses and the other three females produced one, two, and three batches, respectively.

##### **2005 spawning**

Embryos from 37 egg masses produced by two males and five females were genotyped to determine parentage. Due to the atypical results found in 2004 (*i.e.* monogamous matings in nearly all instances), we again elected not to exhaustively genotype the egg masses for potential male contributions. Four random embryos from each egg mass were genotyped to determine maternity in order to assess fecundity and egg energy content for individual females. All of the females spawned in captivity, producing 6-12 egg masses per female, and all mated with both males in the tank. One male (#30) was the apparent sire of 13 egg masses, the other male (#31) of 24. There was no apparent male choice by four of the five females, who mated with both males almost equally. However, one male apparently sired 11 of 12 egg masses with the remaining female.

##### **2006 spawning**

Parentage was assessed for 27 egg masses produced by two males and four females. These were the same parents used in 2005 excepting one female that had died. As before, four random embryos were genotyped, primarily to evaluate maternity for subsequent

assessment of fecundity and egg energy content of individual females. Male contribution was more skewed than in 2005, with Male 31 apparently siring 24 of the 27 egg masses. Females produced 6-9 egg masses, the largest number produced by the same female who spawned the most in 2005. Two females apparently mated with only one male (#31). Of the two females mating with both males, male (#31) apparently sired five of six egg masses with one female and seven of nine with the other.

## Large tank results

### 2005

Developing embryos from 17 Atka mackerel egg masses were collected from two territories defended by males at three different times (Table 3). DNA was extracted from 96 embryos from each egg mass and microsatellite nuclear markers were screened to determine parentage. All genotyped embryos were successfully assigned a male and female parent from the tank. In all cases, a single guardian male fertilized all eggs in its territory, including (in one case) those sampled two weeks apart. Preliminary results (Table 3) indicate a polygynous mating system with up to five females depositing eggs within a territory guarded by a single male. None of the egg masses contained eggs from two different females that were physically adhered.

Egg mass	Territory	Collection Date	Male #	Female#
B	1	8/25/2005	25	9
C	1	8/25/2005	25	14
J-1	1	8/25/2005	25	22
J-2	1	8/25/2005	25	23
I-1	1	8/25/2005	25	14
I-2	1	8/25/2005	25	16
I-3	1	8/25/2005	25	18
I-4	1	8/25/2005	25	19
I-5	1	8/25/2005	25	23
O	1	9/6/2005	25	14
Q	1	9/6/2005	25	14
R	6	10/14/2005	8	23
U	6	10/14/2005	8	24
X-1	6	10/14/2005	8	9
X-2	6	10/14/2005	8	13
Y	6	10/14/2005	8	19
Z	6	10/14/2005	8	23

Table 3. Parentage analyses of embryos from the large tank in 2005.

### 2006

Developing embryos from six egg masses, retrieved from the territory of a single male who had successfully spawned in 2005, were genotyped to determine parentage (Table 4). In all cases, the guardian male was the sole sire of eggs within his territory.

Egg mass	Collection Date	Male #	Female#
1		8	11
2		8	19
3		8	22
4		8	19
5		8	24
6		8	18

Table 4. Parentage analyses of embryos from the large tank in 2006.

Results from captive fish supported our initial null hypothesis, *i.e.* mating is random and involves one fish of each gender in Atka mackerel. Captive males appeared to hold territories for some time period (at least several weeks) successfully monopolizing all matings within their territories and exhibited the largely polygynous strategy documented in other greenlings. However, these results (simple polygyny) were very discordant with results obtained from genotyping partial egg masses retrieved from the stomachs of adult cannibals (see evaluation of egg cannibalism below), that inferred multiple parentage (both male and female) of egg masses. The discrepancies between natural and captive populations may be related to habitat availability and fish densities in the captive environment.

#### **4) Evaluate egg cannibalism using genetic techniques.**

Atka mackerel are distributed in areas of high current in the Aleutian Island chain in Alaska. Males guard nests of batches of eggs at depth from 30-100 meters. Due to the remote areas and high currents of Atka mackerel spawning habitat, egg masses are difficult to obtain in the field. However, adult Atka mackerel caught with trawl gear during NMFS surveys have been found to cannibalize eggs. Therefore, gut contents of Atka mackerel adults were used to obtain samples of egg masses for DNA analyses, both to examine patterns of parentage and cannibalism by both males and females. Individual eggs fuse together into an egg mass once spawned and different egg masses are easily distinguished from each other even when found inside the stomach of adult Atka mackerel. Therefore fused eggs found inside adult stomachs were considered one egg mass presumably spawned at the same time in one location.

Highly polymorphic DNA markers were used to assess parentage of embryos consumed by five adult Atka mackerel of each gender. Gut contents contained both single eggs and one or two partial egg masses (Table 5). Most of these egg masses consisted of batches of developing full and half-sib embryos produced by multiple (3 - 8) parents. All female cannibals were excluded as the mother of embryos they had eaten, thus indicating heterocannibalism. Similarly, four of five males in spawning coloration were excluded as sires of cannibalized embryos, with the exception of one male cannibal, determined to be the sire of two half-sib families detected in one egg mass, thus documenting filial cannibalism by male Atka mackerel. However, this single male sired only 22 % of the embryos genotyped in the egg mass, which contained a minimum of eight parental genotypes. It is unknown if he was the guardian male or an adjacent territory holder. The complex polygamous mating system inferred from preliminary analyses of partially cannibalized egg clutches suggest that sneaked fertilizations and nest raiding by males

may be common behaviors in this species. It seems highly improbable that females would exhibit filial cannibalism and we will focus on parental determination of 10 partial egg masses from male cannibals to get a better estimate of the frequency of this behavior in natural populations.

Cannibal	# egg masses	minimum # of parents			cannibalism type H = heterocannibalism F = filial cannibalism
		mass 1	mass 2	single eggs	
♂ 1	2	8	4	?	H, F. ♂ 1 sired two half-sib families (22% of all cannibalized embryos)
♂ 3	single eggs			?	H
♂ 5	single eggs			?	H
♂ 6	2	3	3		H
♂ 8	2	2	3		H
♀ 1	2	6	5		H
♀ 3	1	8			H
♀ 4	single eggs			2	H
♀ 5	single eggs			6	H

Table 5. Genetic assessment of patterns of cannibalism in male and female Atka mackerel.

Genotypic data from cannibalized embryos were analyzed with the program GERUD 2.0 (Jones 2005). The program is designed to analyze progeny arrays known to have a single mother (or a single father) and both parental genotypes may be unknown. It calculates the most likely parental genotypes based upon allele frequencies in the parental population. Since we currently have allele frequencies for over 600 fish at several locations throughout the Aleutian Islands (M. Canino, unpublished data), this method should have been very successful in reconstructing male genotypes from what were assumed to be egg masses containing half-sibs (*i.e.* single mother, multiple fathers). However, analyses of all cannibalized egg masses exhibiting multiple parentage revealed that at least two females were represented in each egg mass. In other words, a single maternal genotype could not be resolved within a partial egg mass even though it was completely adhered together and there were no discernable boundaries within it. This raises the possibility that female Atka mackerel deposited their eggs upon existing egg masses, fusing the reproductive output of two (or more) females (and perhaps males) into a single mass that no longer consisted of full or half-siblings.

These results stand in stark contrast with those obtained from captive populations indicating a polygynous mating system with a small degree of parasitic fertilizations by males. The deposition of eggs by females onto existing egg masses has not been described in other greenlings but it is a type of behavior that is not likely to be observed or recognized. The only other molecular assessment of the mating system in hexagrammid fishes was conducted on lingcod, where females spawn all of their seasonal reproductive output in a single egg mass (Withler et al. 2002). In this species, guardian males mated with multiple females and parasitic 'sneaked' fertilizations by other males

was common. We have genotyped additional cannibalized partial egg masses and complete egg masses obtained from trawls to further investigate patterns of egg deposition and fertilization in natural populations.

5) *Investigate spatio-temporal variation in reproductive output (maturity schedule, realized fecundity).*

Results of our study of spatio-temporal variability in maturity schedule was presented at the Marine Science in Alaska: 2007 Symposium in January 2007, Anchorage, Alaska. A manuscript is in revision.

A manuscript entitled “Can post-ovulatory follicles be used to distinguish mature from immature Atka mackerel, *Pleurogrammus monopterygius*?” is in internal review and a draft is attached.

6) *Determine fecundity and egg caloric content of successive batches spawned in captivity.* Egg energy was measured using a semi-micro bomb calorimeter for 40 egg masses deposited by five females in the “small tank” at the Seward SeaLife Center in 2005. . Maternity of egg masses was assessed using microsatellite DNA markers (see Parentage section of this progress report, objective 3, small tank results 2005). Preliminary data analysis shows that the amount of energy per egg varies significantly by female (ANOVA,  $p=0.0004$ ,  $df=4$ , figure 3).

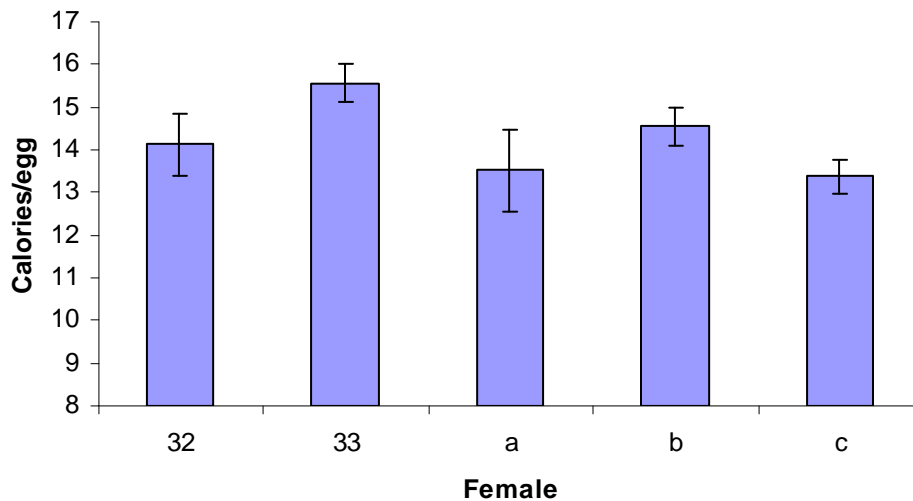


Figure 3. Mean egg energy content for egg masses produced by five females at the Alaska SeaLife Center in 2005.

Energy per egg decreased over the course of the spawning season, (Figure 4), which may be caused by egg energy decreasing in female's later batches. Egg energy decreased for the later batches in some of the females (Figure 5), which is similar to cod (Ouellet et al., 2001). There is a significant interaction between female and batch order ( $p=0.02$ ,  $df=4$ ) implying batch order is a significant factor in egg energy for females. A complete data analysis using maternal length and weight is ongoing. We have begun estimating energy content using the same methodology for the 27 egg masses spawned in 2006 at the Alaska SeaLife Center (see small tank results 2006, objective 3).

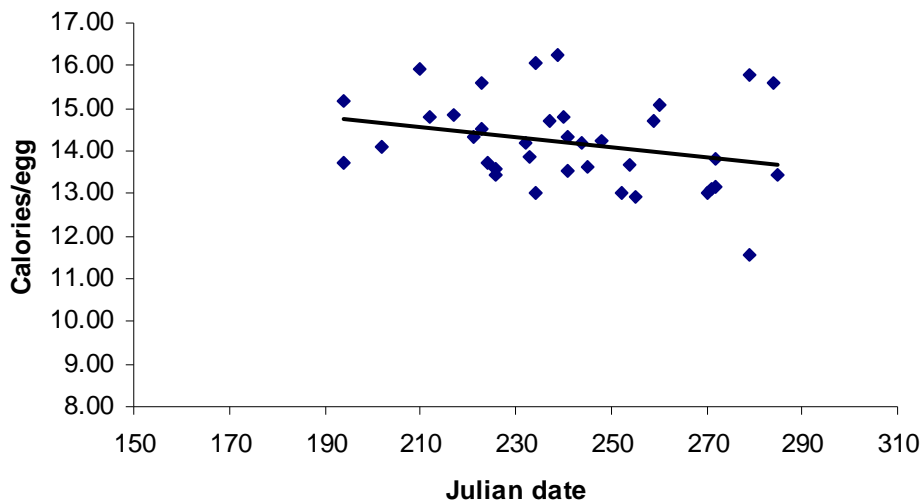


Figure 4. Egg energy (calories per egg) and linear trendline for egg masses produced at the Alaska SeaLife Center in 2005.

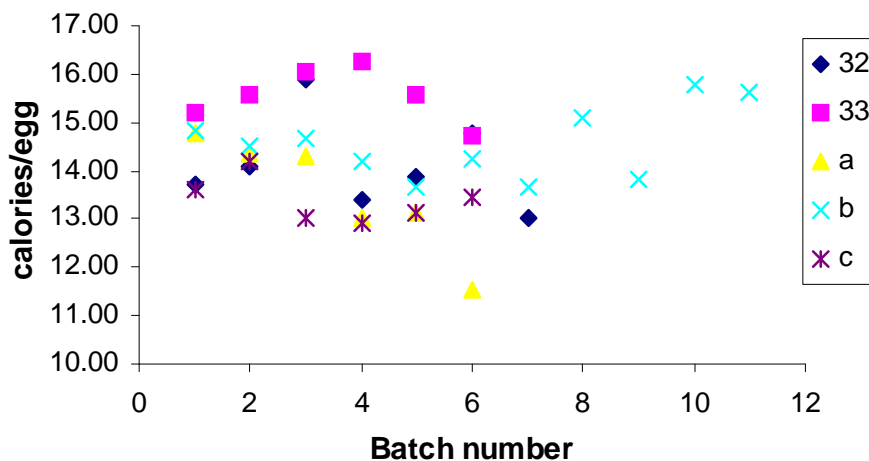


Figure 5. Egg energy by batch order by female for egg masses collected at the Alaska SeaLife Center in 2005.

A method to measure egg energy from ovaries removed from wild collected fish has been developed. Ovaries were removed from 208 females during a sampling cruise aboard the F/V Seafisher during spawning season in the Aleutian Islands, Alaska in 2005. One ovarian lobe for each ovary was stored in formalin for histological analysis, and the other lobe frozen for later measurement of egg energy. The lobes stored in formalin have been histologically processed and maturity stage has been assigned. Eighty females with hydrated eggs have been identified and batch order of the hydrated eggs has been assigned into three categories using histology: first batch, middle batch, or last batch. Egg energy has been measured with a bomb calorimeter for 12 of these wild collected females. There is a high correlation between egg dry weight and egg energy content for the wild collected fish ( $r^2=0.9787$ ) and the pooled wild and AFSC collected fish ( $r^2=0.9662$ ) (figure 6). Because of the strong relationship found between egg dry weight and egg energy content, we plan to measure egg dry weight for the remaining 68 wild collected samples with hydrated eggs to determine: 1) If females in the wild produce eggs with lower energy content than females which consume more food in captivity and 2) If maternal size and age affect egg energy content in the wild.

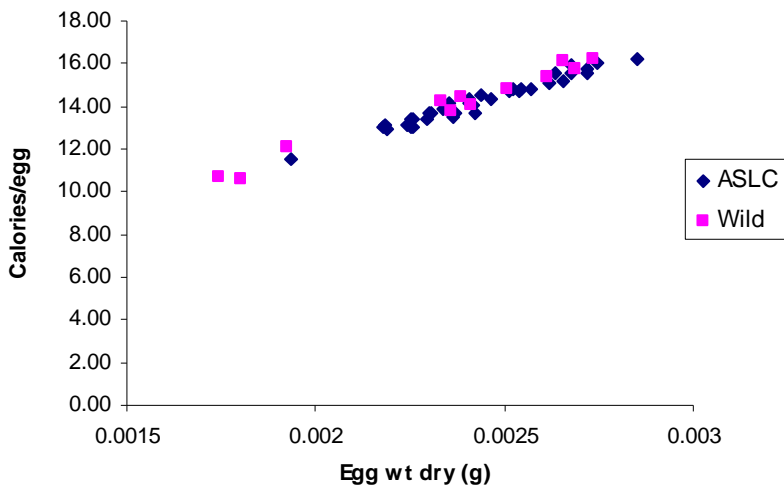


Figure 6. Egg energy compared to egg dry weight for egg masses spawned at the ASLC in 2005 and for hydrated eggs removed from ovaries collected in the wild in 2005.

Fecundity has been estimated for the 40 egg masses collected during 2005 and the 27 egg masses collected during 2006. Two of the batches collected in 2005 were combined into one batch because they were spawned the same day by the same parents. The five females in 2005 spawned a mean number of 7.8 batches, which is higher than the 6.13 estimated number of batches spawned by wild fish (McDermott et al. 2007). Female

lengths and weights at the Alaska SeaLife Center were collected. Female fecundity will be compared between these results and the fecundity of fish caught in the wild.

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Ouellet P, Lambert Y, Berube I. 2001. Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. ICES J. Mar. Sci. 58: 672-686.

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Withler RE, King JR, Marliave JB, Beath B, Li S, Supernault KJ, and KM Miller. 2004. Polygamous mating and high levels of genetic variation in lingcod, *Ophiodon elongatus*, of the Strait of Georgia, British Columbia. Env. Biol. Fish. 69:345-357.

## Outreach:

Spies, IS, (2007). It's elementary dear Watson, why DNA is a useful tool for detectives and fish biologists. Presentation to fisheries high school students, teachers, and principal, at St. Paul School, AK April 27, 2007

McDermott S. F. , Cooper, D. C., Guthridge J. R. Lauth, E. A. Logerwell, N. Hillgruber, K. Rand (2007). Atka mackerel, Sea lions, and trawl exclusion zones: a look below the surface. Departmental seminar, University of Alaska, Fairbanks, School of Fisheries, Juneau. April 2007.

Canino MF, Spies IS, McDermott S, Atkinson S, and Guthridge J. 2007. Captivity effects on the mating behavior of Atka mackerel? Marine Science in Alaska: 2007 Symposium. January 2007, Anchorage, Alaska. (Poster)

Cooper D. W. and McDermott S. F. (2007). Reproductive ecology of Atka mackerel: Variability in Atka mackerel length and age at maturity. Marine Science in Alaska: 2007 Symposium. January 2007, Anchorage, Alaska (Oral presentation).

Guthridge, J. L., N. Hillgruber, and R. Lauth (2006). Embryonic development of Atka mackerel and the effect of temperature. AFS Juneau Student Symposium. April 2006 (Oral presentation)

Guthridge, J. L., N. Hillgruber, and R. Lauth (2006). The effect of temperature on hatch time for Atka mackerel. 14<sup>th</sup> Western Groundfish Conference, Newport, Oregon. January/February 2006 (Poster)

Guthridge, J. L., N. Hillgruber, and R. Lauth (2006). The effect of temperature on hatch time for Atka mackerel. Marine Science in Alaska: 2006 Symposium. January 2006, Anchorage, Alaska. (Poster)

Cooper, D., and S. F. McDermott (2006). Atka mackerel reproductive biology, Temporal and spatial variation in Atka mackerel maturity schedule. Marine Science in Alaska: 2006 Symposium. January 2006, Anchorage, Alaska. (Poster)

McDermott S. F.(2006). Current research of Atka mackerel at the Alaska Fisheries Science Center. Multicultural Initiative in the Marine Sciences (MIMSUP event), Alaska Fisheries Science Center.

Spies, I.S. and M. Canino. (2006) Atka mackerel reproductive biology, Genetic assessment of cannibalism and the mating system of Atka mackerel. Marine Science in Alaska: 2006 Symposium. January 2006, Anchorage, Alaska. (Poster)

Spies, I.S. and M. Canino. (2006) "Its elementary, dear Watson"; How genetics can provide clues to population structure, cannibalism, and the mating system of Atka Mackerel. Science in the Sound Speaker Series, Highline Community College. May, 2006, Seattle, WA. (Oral presentation; [http://flightline.highline.edu/mast/06-Speaker\\_listing.htm](http://flightline.highline.edu/mast/06-Speaker_listing.htm)).