Reproductive biology and ecology of Pacific hagfish (*Eptatretus stoutii*) and Black hagfish (*Eptatretus deani*) off the coast of Vancouver Island, BC

by

Aharon Fleury
Bachelor of Science, University of Hawai`i at Mānoa, 2013

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

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Abstract

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Hagfish are one of the more lucrative commercial opportunities in the world with fisheries harvesting them specifically for food and for their skin for leather-based products. In 2013 a three year experimental fishery opened off the west coast of Vancouver Island, British Columbia in order to determine the sustainability of a Pacific hagfish (Eptatretus stoutii) and Black hagfish (Eptatretus deani) fishery. In this study, I examined the reproductive biology of both species including: length-weight relationships, sex ratios, fecundity, and size-at-gonadal development. This study corroborates previous suggestions that Pacific hagfish are juvenile protogynous hermaphrodites while black hagfish are likely to be dioecious with an unknown juvenile stage. Sexual dimorphism appears in both species of hagfish, which is likely the result of inter- and intraspecific morphological differences. For both species fecundity decreases throughout developmental stages, and the average fecundity is very low (27-32 eggs per female). Additionally, in both species females commence gonadal development prior to males. Furthermore, both species exhibited extreme female: male sex ratios across length-classes, however, in opposite directions. The reproductive biology of Pacific and Black hagfish models that of a $k$-selected species, which is a species that tends to live long and has a slow growth rate, low fecundity, and late maturity. As a result there are a variety of concerns that should be addressed when developing a sustainable hagfish fishery to prevent collapses observed in previous hagfisheries.
Table of Contents

Supervisory Committee .......................................................... ii
Abstract .................................................................................. iii
Table of Contents ...................................................................... iv
List of Tables ........................................................................... v
List of Figures ........................................................................... vi
Acknowledgments ...................................................................... vii
Chapter 1 - Introduction ............................................................. 1
  1.1 Thesis structure ............................................................... 1
  1.2 Overview ........................................................................... 1
    1.2.1 Importance of Hagfish Fisheries ..................................... 1
    1.2.2 Ecology of hagfish ......................................................... 3
    1.2.3 Reproduction and growth of hagfish ............................... 5
    1.2.4 Sexual differentiation .................................................... 6
    1.2.5 Hagfish Fisheries ........................................................... 7
  1.3 Objectives and justification for research ............................... 8
    1.3.1 Objectives .................................................................. 8
Chapter 2 – The reproductive biology and ecology of Pacific hagfish (Eptatretus stoutii) and Black hagfish (Eptatretus deani) off the coast of Vancouver Island, BC ........................................ 10
  2.1 Understanding reproductive biology for a sustainable fishery .... 10
  2.2 Methods ................................................................. 13
    2.2.1 Hagfish dissection and reproductive stages ..................... 14
    2.2.2 Statistics and fisheries equations ..................................... 15
  2.3 Results .................................................................. 16
    2.3.1 Length-weight relationship .......................................... 16
    2.3.2 Sex ratio ................................................................. 17
    2.3.3 Fecundity ................................................................. 18
    2.3.4 Development curves .................................................... 20
  2.4 Discussion ................................................................ 20
    2.4.1 Review of reproductive terminology and sex determination in E. stoutii and E. deani .............................................................. 21
    2.4.2 Length-weight relationships across fisheries ................... 22
    2.4.3 Sex ratio and development curves ................................ 25
    2.4.4 Comparing fecundity between species and fisheries .......... 27
    2.4.5 Age estimation .......................................................... 29
    2.4.6 Seasonal distributions ................................................. 30
  2.4.7 Conclusion ............................................................... 30
Bibliography ............................................................................ 33
Figures .................................................................................. 38
Tables .................................................................................... 46
List of Tables

Table 1. Stages of sexual differentiation for both E. stoutii and E. deani. The methodology was adapted from Gorbman (1990) and Martini and Beulig (2013). The descriptions are based on macroscopic observations. The female stages are cyclical whereas the male stages are linear over their lifetime.

Table 2. Mean length (mm), standard deviation (SD), and range for both E. stoutii and E. deani. No simultaneous hermaphrodites were found in E. deani samples.

Table 3. Post-hoc Tukey HSD comparison test comparing mean lengths against sex (F= Female (Stages 1 to 8), M= Male (Stages -1 to -4), U= Unknown (Stage 0), H= Hermaphrodite (Stage 9)) within species (Pacific, Black) and the interaction between sexes and species. Means with the same letter in superscript are not significantly different.

Table 4. Average fecundity ± standard deviation for all females 3+ of each species and for reproductive stages with less than 150 eggs (3-8). E. deani average fecundity was always greater than E. stoutii except for Stage 8, however it was not always significant.
List of Figures

Figure 1. A) A quadratic curve \((\log W = a + bL + cL^2)\) of log weight versus total length for \(E. stoutii\); \(a = -6.530e^{-02}\), \(b = 1.610e^{-02}\), \(c = -1.122e^{-05}\) ................................................................. 38

Figure 2 Seasonal comparison of length-weight relationships for \(E. stoutii\). In larger hagfish the hagfish are heaviest in the winter and lightest in the spring. In smaller hagfish the hagfish are heaviest in summer and lightest in fall ................................................................. 39

Figure 3 A) \(Eptatretus stoutii\) comparison of male and female length-weight curves using the standard fisheries equation \(W = aL^b\). The curves are significantly different with males heavier than females. ................................................................. 40

Figure 4 A) Total length compared to reproductive stages for \(E. stoutii\). ........................................ 41

Figure 5 A seasonal comparison of reproductive stages for \(E. stoutii\). A similar pattern is observed across all seasons ................................................................. 42

Figure 6 A) \(Eptatretus stoutii\) sex ratios (female: male) as a function of total length sorted into 25 mm total length bins. The mean sex ratio was 1.47 with a range of 50:1 at 250-274 mm to 0.7:1 at 500-524 mm................................................................. 43

Figure 7 A) Fecundity as a function of total length. There is a significant relationship for \(E. stoutii\) (left), but no significant relationship for \(E. deani\) (right). ................................................................. 44

Figure 8 Sexual development curves for \(E. stoutii\) (left) and \(E. deani\) (right). The data were sorted into 50 mm bins. Each curve used a variation of the three-parameter logistic equation: \(Percent = 100/(1+(TL_{50}/X)^b)\). ................................................................. 45
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Chapter 1 - Introduction

1.1 Thesis structure
Chapter 1 provides background on the ecology and reproductive biology of hagfish. It also includes a brief review on the history and purpose of hagfish fisheries in Japan and Korea and the eventual introduction of hagfish fisheries in North America. Finally, it introduces the interest for a Pacific (*Eptatretus stoutii*) and Black hagfish (*Eptatretus deani*) fishery in British Columbia and the goals set out for a new experimental hagfish fishery.

Chapter 2 investigates the reproductive biology using reproductive stages and length-weight relationships of Pacific and Black hagfish in British Columbia. Furthermore, length-weight relationships are compared to older hagfish fisheries in the Northeast Pacific ocean. Inter- and intraspecific variation is examined for a variety of variables including: length, length-weight relationships, fecundity, egg length, and sex ratios. Additionally, development curves were utilized to identify size-at-development for females and male hagfish of both species.

1.2 Overview

1.2.1 Importance of Hagfish Fisheries
Hagfish belong to what is regarded as one of the most primitive classes of fish in the world, Agnatha. Their primitive characteristics have attracted research attention resulting in a plethora of information regarding their physiology and biochemistry, but little information about their reproductive biology and ecology (Leask and Beamish 1999). They are separated into five genus groups across the world but only two are primarily discussed due to their abundance, *Myxini* and *Eptatretus*. Although 83 species
of hagfish exist only two species inhabit the west coast of North America, Pacific hagfish (*Eptatretus stoutii*) and Black hagfish (*Eptatretus deani*) (Hart 1973, Gorbman 1990, Ellis et al. 2015). Both species of hagfish span extensive ranges across the northeast Pacific Ocean. Pacific hagfish range from Pt. San Pablo, Baja California to southeastern Alaska at depths of 16 m to 633 m (Wisner and Mcmillan 1990). Black hagfish range from Cedros Island, Baja California to southeastern Alaska at depths of 107 m to 2743 m (Wisner and Mcmillan 1990). Hagfish are regarded as one of the most abundant demersal fish species in terms of abundance and biomass (Wakefield 1990).

Hagfish have been popular in South Korea and Japan since World War II due to demand for their eel-skin leather and meat (Strahan and Honma 1960, Gorbman et al. 1990). In the 1980s, Korean and Japanese hagfish fisheries began to collapse due to decreasing biomass of Japanese hagfish (*Eptatretus burgeri*) (Gorbman et al. 1990). As a result, in 1988 hagfish fisheries targeting *E. stoutii* and *E. deani* opened along the California, Oregon, and British Columbia coasts to export hagfish to South Korea (Gorbman et al. 1990, Barss 1993, Leask and Beamish 1999). However, the new fisheries were only open for intermittent intervals of time due to fluctuations in demand in South Korea resulting in their closure in 2001 (Benson et al. 2001). Nonetheless, a renewed interest in hagfish fisheries in British Columbia has developed resulting in the creation of an experimental hagfish fishery off the west coast of Vancouver Island from 2013 – 2016. Furthermore, prior to expanding the experimental fishery to a full-scale commercial fishery the reproductive biology, abundance, and ecology of *E. stoutii* and *E. deani* must be assessed in order to guarantee a sustainable fishery.
1.2.2 Ecology of hagfish

All species of hagfish are exclusively marine and reside in various types of benthic habitats. Atlantic hagfish, *Myxine glutinosa*, are found in the Atlantic and have been found to be well-adapted to hypoxic environments likely due to their highly vascularized skin (Lesser et al. 1996). It is likely that other species of hagfish such as *Eptatretus* spp. have similar biochemical and physical adaptations to survive hypoxic conditions. Hagfish are predominately found near mud and other soft substrates and have been observed burrowing in the substrate (Cailliet et al. 1991, Leask and Beamish 1999). However, Pacific and Black hagfish have also been found residing in areas of mixed substrates like sand and gravel or sand and boulders, but appear to prefer mud bottoms (McInerney and Evans 1970, Pearcy et al. 1989, Barss 1993).

Other abiotic factors in addition to substrate, such as temperature and salinity, appear to have an effect on hagfish distribution. Water temperature is the primary limitation for hagfish habitats (Powell et al. 2005). Hagfish have not been found in waters warmer than 22°C suggesting that this is their maximum thermal tolerance (Martini 1998). Furthermore, along the coast of Vancouver Island in Barkley Sound, Pacific hagfish reside in water temperatures of approximately 10°C suggesting that they prefer colder temperatures (McInerney and Evans 1970). Hagfish prefer high salinity waters, approximately 31-32 ppt, and become agitated when the water is diluted (McInerney and Evans 1970).

Hagfish are slow swimmers and remain in relatively isolated populations resulting in smaller home ranges (Martini 1998). Only one species of hagfish, *E. burgeri*, is known to have seasonal migrations across multiple depths, but other species exhibit possible seasonal movements as well (Wakefield 1990, Gorbman et al. 1990). For instance,
Stephens (1995) observed that the population of Pacific hagfish off the Californian coastline migrate inshore to spawn from December through March. This migration resulted in an increased inshore abundance of smaller individuals such as females and juveniles, which usually occupy deeper waters. There was a return migration of the same population of smaller individuals to deeper waters from April through November leaving larger males inshore. Thus, it has been suggested that habitat partitioning could be the result of differing ages and sex (Stephens 1995). In a similar study in Monterey Bay, California, large male Pacific hagfish were located at shallower depths of approximately 100 m while the females were found at deeper depths, with smaller individuals at intermediate depths of approximately 250 m (Johnson 1994). Furthermore, intraspecies habitat partitioning appears to occur in hagfish. For example, in contrast to Pacific hagfish, small Black hagfish are found at depths of 750 m while larger individuals are found deeper (Johnson 1994). Nevertheless the main reasons for their habitat partitioning and seasonal migrations are not well understood but are likely related to a wide range of physical and biological parameters that may fluctuate with depth (Bailey et al. 2007).

Hagfish play an important role in benthic and bentho-pelagic communities. Hagfish are primarily scavenging species and have been observed feeding on whale carcasses, dogfish, cod, salmon, and other carrion (Hart 1973). Besides scavenging, it is thought that hagfish may be opportunistic feeders that prey on small fish and invertebrate species (Martini 1998). Stomach analyses found *E. stoutii* and *E. deani* prey on mesopelagic organisms such as shrimp, small fish, cephalopods, and benthic polychaetes (Johnson 1994). Unfertilized eggs have also been observed in hagfish guts (Worthington 1905). Hagfish also play an important role in their community by recycling and
maintaining the sea bottom via scavenging and burrowing into the substrate and further releasing organic material into the benthos (Martini 1998). Hagfish are also a common prey species of cod, dogfish, seals, porpoises, sea lions, dolphins, octopus, and harbor seals (Martini 1998). Additionally, hagfish are thought to be strong competitors within benthic communities due to the mucus they produce while feeding. Research has shown that other fish have succumbed to hagfish mucus during mass feedings on carrion as a result of mucus becoming lodged in their respiratory system (Davies et al. 2006).

1.2.3 Reproduction and growth of hagfish

There is very little information on reproduction and growth for most species of hagfish. All life stages in hagfish are thought to be free-living, meeting their food requirements through scavenging and predation (Johnson 1994). In general, hagfish are slow growing (approximately 4-5 cm per year) and have low fecundity ranging from 1 to 30 eggs per female (Nakamura 1994, Leask and Beamish 1999). Currently, growth and maturity estimates only exist for *E. burgeri* and *E. stoutii* (Patzner 1978, Nakamura 1994). *Eptatretus burgeri* spawn annually, and females reach reproductive maturity at around 9-10 years indicating a very low reproductive potential (Patzner 1978). Late maturity was also observed in *E. stoutii* with maturation estimated to occur at 325 mm or 8-12 years of age (Nakamura 1994). Seasonal patterns for spawning migration have also only been observed in Japanese hagfish; however, similar spawning patterns have been suggested for Pacific hagfish though not confirmed (Walvig 1963, Kobayashi et al. 1972, Patzner 1998).

Pacific hagfish have an average fecundity of 28, and are thought to spawn in the summer (Barss 1993). This seasonality was also observed in the California fishery with
females and juveniles remaining offshore outside of the spawning season, and males remaining inshore (Stephens 1995). However, these seasonal patterns were not observed in British Columbia populations (Benson et al. 2001). Nonetheless, hormone analyses suggest hagfish without obvious seasonal patterns, such as the populations in British Columbia, may have a reproductive seasonal cycle that has yet to be identified (Powell et al. 2005). Thus, most of our knowledge of the reproductive characteristics of hagfish is preliminary or non-existent, particularly for *E. stoutii* and *E. deani*.

### 1.2.4 Sexual differentiation

One of the many challenging issues regarding hagfish biological traits revolves around their sex determination, which is often different across species. For instance, there was debate as to whether Pacific hagfish are protandrous hermaphrodites, dioecious with an undifferentiated juvenile stage, or dioecious with a hermaphroditic larval stage (Nansen 1887, Schreiner 1955, Jensen 1966). Gorbman (1990) ended this debate with histological analyses concluding that Pacific hagfish are juvenile protogynous hermaphrodites. However, the epigenetic factor causing the ontogenetic shift in gender was not determined (Gorbman 1990). Gorbman (1990) found that the long gonadal fold first develops ovarian tissue in the anterior portion of post-larval hagfish and that testicular tissue later develops in the posterior portion of some individuals usually around 240 mm in size. Furthermore, in larger Pacific hagfish there was a low incidence of simultaneous hermaphroditism (0.03 – 0.23%) (Barss 1993, Johnson 1994). No histological analyses have been performed on Black hagfish, however, Powell et al. (2005) suggested that many species of hagfish may be functional sequential hermaphrodites that also function separately as males and females. Sex determination
remains one of the major issues of concern in fisheries due to the requirement of using hagfish sex in estimating other biological parameters.

1.2.5 Hagfish Fisheries

The hagfish fishery has been prominent in Asian waters for decades as hagfish were primarily used for soft leather and food in Japan and South Korea (Gorbman et al. 1990, Honma 1998). The increase in demand for hagfish has resulted in depleted hagfish fisheries. In Japan the fisheries in the Nigata area and Sado Strait have declined significantly to the point where commercial fisheries are no longer viable (Gorbman et al. 1990, Powell et al. 2005). As a result, there has been a shift towards targeting Pacific and Black hagfish in the NE Pacific Ocean (Gorbman et al. 1990). Unfortunately, no abundance estimates currently exist for many of the stocks along the west coast of North America, including BC populations (Benson et al. 2001).

Other fisheries along the west coast of North America such as the California fishery appear to be in decline due to lack of regulations as a result of a gap in information regarding hagfish reproduction. A new experimental fishery was implemented on the west coast of Vancouver Island in 2013 for the sole purpose of filling these information gaps. This fishery has been part of a long-term effort to determine whether a hagfish fishery is viable for the British Columbia stock.

Prior to the 1960s, hagfish were primarily fished as a food source for both Korea and Japan with limited manufacturing of “eelskin” from the hagfish skin (Strahan and Honma 1960, Gorbman et al. 1990). After 1960 an increase in demand for “eelskin” resulted in an increase in fishing for both countries. The E. burgeri stock biomass slowly declined due to overfishing through the 1980s (Gorbman et al. 1990). By 1988
overfishing of Asian stocks and the demand for “eelskin” resulted in the expansion of new hagfish fisheries in the northeast Pacific Ocean including Baja California, California, Oregon, and British Columbia (Gorbman et al. 1990, Barss 1993, Johnson 1994, Leask and Beamish 1999). These new fisheries targeted the two data-limited Pacific (\textit{Eptatretus stoutii}) and Black hagfish (\textit{Eptatretus deani}).

In British Columbia two experimental fisheries were created, one running from 1988-1992 and another from 1999-2001. Both fisheries targeted Pacific and Black hagfish, however, only Pacific hagfish data were reported (Leask and Beamish 1999, Benson et al. 2001). Samples from those fisheries found that Pacific hagfish can range from 186 mm to 631 mm in length (mean = 400 mm) (Benson et al. 2001). The BC stock was estimated to be mostly mature with a large proportion of hagfish ranging between 15-25 years of age (Leask and Beamish 1999). Additionally, it was observed that Black hagfish were larger than Pacific hagfish (Leask and Beamish 1999). Thus, as a result of the dearth of information from previous BC fisheries, the new 2013-2016 fishery was created to enhance our knowledge of the Pacific and Black hagfish.

1.3 Objectives and justification for research

1.3.1 Objectives

The experimental fishery, which opened off the west coast of Vancouver Island in 2013, was created in part to enhance our understanding of \textit{E. stoutii} and \textit{E. deani} reproductive traits. The objective of this study was to identify hagfish reproductive characteristics in order to determine fecundity, length-at-maturity, length-size frequencies, and length-weight relationships for both species. The reproductive and ecological traits of \textit{E. stoutii} and \textit{E. deani} will allow managers of hagfish fisheries along
the west coast of North America to successfully maintain their hagfish stocks and could potentially be expanded to other species of hagfish around the world.
Chapter 2 – The reproductive biology and ecology of Pacific hagfish (*Eptatretus stoutii*) and Black hagfish (*Eptatretus deani*) off the coast of Vancouver Island, BC

Preface: Please take note that this chapter is written as a stand-alone journal manuscript.

2.1 Understanding reproductive biology for a sustainable fishery

Understanding the life history of a fish population is as important as knowing the biomass when managing a sustainable fishery (Hutchings 2002, Berkeley et al. 2004). Selective pressure due to fishing can reduce genotypic variation and disrupt the spatial distribution within a population preventing the full recovery of a depleted population (Hutchings 2000, Heino and Godo 2002, Berkeley et al. 2004, Juanes et al. 2007). It is especially important to understand basic reproductive characteristics such as fecundity, age-at-maturity, and size-at-maturity in order to minimize fishing mortality of late-maturing species (Brander 1981, Walker and Hislop 1998). Benthic ecosystems, in particular, often consist of these late-maturing species that are highly vulnerable to overfishing (Hutchings 1999, DFO 2000). Furthermore, new research recommends using models which acknowledge fisheries-induced evolution, energetics and reproductive performance, and skipped spawning, which can all affect the reproductive success of a fish (Lowerre-Barbieri et al. 2011). This study will explore the life histories of two hagfish species with the goal of providing information that can help limit overfishing in future hagfish fisheries.

Prior to the 1960s, Japanese hagfish (*Eptatretus burgeri*) were primarily fished in the Sado Straight of the Japan Sea as a food source for both Korea and Japan with limited manufacturing of “eelskin” from the hagfish skin (Strahan and Honma 1960, Gorbman et
al. 1990). After 1960 an increase in demand for “eelskin” resulted in an increase of fishing for both countries’ hagfish fisheries. As a result of the lack of research on the reproductive traits of hagfish species the stock biomass slowly declined due to overfishing through the 1980s (Gorbman et al. 1990). By 1988 overfishing of Asian stocks and the demand for “eelskin” resulted in the expansion of new hagfish fisheries in the northeast Pacific Ocean including Baja California, California, Oregon, and British Columbia (Kato 1990, Gorbman et al. 1990). These new fisheries targeted the two data-limited Pacific (Eptatretus stoutii) and Black hagfish (Eptatretus deani) species.

The Pacific and Black hagfish are two of 83 species of hagfish that make up the most primitive Class of vertebrates, Myxini (Knapp et al. 2011, Martini and Beulig 2013). Both of these hagfish species span extensive ranges across the northeast Pacific Ocean. Pacific hagfish range from Pt. San Pablo, Baja California to southeastern Alaska at depths of 16 m to 633 m (Wisner and Mcmillan 1990). Black hagfish range from Cedros Island, Baja California to southeastern Alaska at depths of 107 m to 2743 m (Wisner and Mcmillan 1990). In British Columbia, the new hagfish fisheries constantly fluctuated, open through intermittent periods from 1988-1992 and 1999-2001, due to changes in demand and the reluctance to overfish given the dearth of reproductive and length/weight data available for both species (Benson et al. 2001). However, even with the new fisheries efforts, only limited reproductive data have been collected for a few species of hagfish, dominated by the Japanese hagfish (Eptatretus burgeri) (Kobayashi et al. 1972, Ichikawa et al. 2000, Nozaki et al. 2000) and secondarily by Pacific hagfish (Gorbman 1990, Barss 1993, Johnson 1994, Benson et al. 2001).
A still unresolved issue which impacts hagfish fisheries is the differentiation of sexes in both *E. stoutii* and *E. deani*. Previous research suggested that these hagfish were either protandrous hermaphrodites, juvenile hermaphrodites, or dioecious (Gorbman 1990). After extensive histological examination Gorbman (1990) determined that *E. stoutii* are juvenile protogynous hermaphrodites becoming sexually differentiated males and females after maturity with the incidence of adult hermaphroditism only 0.3% of the population. The only other study on this species appears to be in agreement with this conclusion (Johnson 1994). Thus far, no histological examination has been performed on *E. deani*. In both species mature females have a low fecundity of 1-30 eggs with no seasonal or annual cycles identified, and a sex ratio of approximately 1.8:1 (female to male) and 2.58:1, Pacific and Black respectively (Gorbman 1990, Johnson 1994, Leask and Beamish 1999). Size differences also occur between species and sexes. In general Black hagfish are larger than Pacific hagfish (Barss 1993, Johnson 1994, Leask and Beamish 1999). Pacific females are smaller than the males while Black females are larger than the males (Barss 1993, Johnson 1994, Leask and Beamish 1999). The differences in size between species has been attributed to food availability at different depths (Johnson 1994).

An experimental fishery opened off the west coast of Vancouver Island in 2013, and was created in part to enhance our understanding of *E. stoutii* and *E. deani* reproductive traits. The objective of this study was to quantify hagfish reproductive characteristics in order to determine fecundity, length-at-maturity, length-size frequencies and length-weight relationships. Better knowledge of the reproductive and ecological traits of *E. stoutii* and *E. deani* will allow managers of hagfish fisheries along the west
coast of North America to successfully maintain their hagfish stocks and could potentially be expanded to other species of hagfish around the world.

2.2 Methods

In 2012 Lenico Holdings Inc received the only permit to fish for *Eptatretus stoutii* and *Eptatretus deani* off the west coast of Vancouver Island in an experimental fishery. The experimental fishery is set to run from 2013-2016 with possible extensions based upon survey results. Under the permit three survey types are required: depth-stratified, depletion, and a biological index site at Kirby Point (PFMA 23-7). Two fishing vessels, Ocean Marauder and Viking Sunrise, collected samples from multiple Pacific Fishing Management Areas (PFMA), specifically targeting PFMAs 23/123, 25/125, 08/108 - 09/109, which represent high, medium, and low effort areas respectively. Dependent on the survey different traps were utilized. For depth-stratified and depletion surveys, 25x 227L barrels with 16 mm escape holes were spaced 25 fathoms apart for one set. For consistency with the earlier fisheries, Korean traps, thin, long traps with 16 mm holes, were used to survey Kirby Point and gather biological data. Each set of traps was filled with commercial bait and ranged in soak time from 4-48 hrs. After specified soak times the traps were hauled on board with the observer randomly selecting approximately 100 hagfish per set to freeze for dissection. In the case of F/V Ocean Marauder samples, the hagfish removed for biological data were frozen on board in ~25 kg bags. The F/V Viking Sunrise had no onboard freezer, so hagfish were kept cool in tanks and then frozen after offloading in the harbor. The frozen bags of hagfish from the early years of the experimental fishery remained frozen for 1-2 years until July 2014 when we began dissections.
2.2.1 Hagfish dissection and reproductive stages

The 25 kg frozen bags of hagfish were removed individually from industrial freezers for dissection. The hagfish were removed and submerged in a flooded sink of cold water to thaw for 2 to 3 hours. After thawing, individual hagfish were pulled off the large block of fish to remove excess frozen slime and waste. The hagfish were individually weighed (wet weight, ±0.01 g), measured (±0.1 cm), and dissected to determine sex and reproductive stage. Gonadal tissue was removed and weighed (±0.01g) in select cases; however, due to extended freezing the intestine often burst preventing clean removal of gonadal tissue. Reproductive stages were determined after a brief histological examination based on two methods outlined by Martini and Beulig (2013) and Gorbman (1990) (see Table 1).

Histological observations were made macroscopically (Table 1). It should be noted that under a microscope Stage 0 hagfish could have been classified into one of 3 categories: undifferentiated, immature female, or sterile (i.e., gonads develop partially resulting in an infertile adult), but in macroscopic inspection all were combined as a Stage 0 hagfish (Schreiner 1955, Gorbman 1990, Martini and Beulig 2013). Male reproductive stages are linear (e.g., with increasing size/age males move from Stage 1 to Stage 4 maintaining Stage 4 reproductive status once achieved for the rest of their lifetime) while female reproductive stages are representative of a cyclical reproductive cycle. Thus, the stages cannot be used to describe maturity in female hagfish, but instead can be used to identify a size at which the first developmental cycle begins.

Each hagfish was dissected starting from the cloaca laterally across the ventral side of the body cavity until reaching the pharyngocutaneous duct. The mesentery
connecting the intestine and muscle wall was then carefully examined to identify reproductive stages. If present, the lengths of three most average-sized eggs were measured with a caliper to calculate an estimated egg length with which to identify the reproductive female stage (Table 1). The total number of eggs was then counted to estimate fecundity. Flaccid postovulatory follicles indicating recent spawning activity (Stage 8, Table 1), which are capsules of recently spawned mature eggs left in the body cavity, were counted to calculate post-spawning average fecundity. A total of 3904 E. stoutii and 303 E. deani were dissected.

2.2.2 Statistics and fisheries equations
We calculated length-weight relationships using the equation \( W = aL^b \), where \( W \) = wet weight, \( L \) = total length, \( a \) = the intercept (fish body form), and \( b \) = the slope (growth coefficient) for both E. stoutii and E. deani to assess and compare condition (Ricker 1973, 1975). The parameters \( a \) and \( b \) were estimated by linear regression analysis on log-transformed data. To compare goodness of fit, a second polynomial non-linear regression was also used to assess length-weight relationships for both species and seasonal length-weight relationships for Pacific hagfish. A three-parameter logistic equation was used, with the y-axis normalized as percent and 100 defined as the maximum, to estimate the percentage of stage 0 hagfish as a function of total length (i.e. TL\(_{50}\)) (Martini and Beulig 2013). Separate female and male curves indicating size-at-gonadal development were generated using the same three-parameter logistic equation under the assumption that the genetic sex ratio is 1:1 (Martini and Beulig 2013). Two-sample t-tests and ANOVA were used to determine significant differences between species, sex, length, and weight. A Tukey HSD test was employed to specify which interaction factors were significant after
significant ANOVA tests. An F-test and an ANCOVA were used to determine sex and seasonal differences in length-weight relationships. All statistical analyses were performed using RStudio with R 3.2.4.

2.3 Results

2.3.1 Length-weight relationship

Length-weight relationships were significant for both species with length strongly predicting weight at a given length (for both *E. stoutii* and *E. deani* p<0.01, $r^2=0.92$, Fig 1A and 1B). We found a significant difference (p<0.01) in length-weight relationships between species, and among seasons (p<0.01) for *E. stoutii* (Fig. 2). We found significant differences between all seasons except between summer and spring. Biological samples for *E. deani* were only available from the summer months therefore no seasonal analyses were possible.

When comparing length-weight relationships between males and females we found contrasting differences across species. Pacific hagfish males were significantly larger than females (p<0.01) (Fig 3A), while the opposite was true for *E. deani* with females significantly larger than males (Fig 3B). Large differences in the y-intercepts suggest a strong sexual dimorphism in *E. deani*.

Table 2 provides information on length for *E. stoutii* and *E. deani*. An ANOVA found no significant overall difference in mean lengths between the species (p=0.31). However, we did detect significant differences when including sex within and across species (p<0.01). A Tukey post-hoc further differentiated differences between sexes and the interaction between species and sex (Table 3). There were significant differences between all sexes in mean lengths for both species. Additionally, the interaction effect
between sex and species found Pacific males and Black females were similar in mean length, however, significantly different from Pacific females and Black males which were also similar in mean length. All hagfish longer than the maximum length of Stage 0s were found to be either a male, female, or hermaphrodite. Thus, we can assume all hagfish have commenced gonadal maturation after the Stage 0 maximum length of 460 mm for *E. stoutii* and 398 mm for *E. deani*.

Figures 4A and 4B show the variability in lengths in reproductive stages for both *E. stoutii* and *E. deani*. The variability in the females for both species is representative of the cyclical nature of the female reproductive cycle, which occurs in all sizes of mature females (i.e. re-cycling through Stages 1-8). In contrast, the variability in males suggests potential differences in male gonadal maturation. The stages of male maturation significantly increased with length for both Pacific (*p*<0.01, $r^2 = 0.48$) and Black hagfish (*p*<0.01, $r^2 = 0.44$) (data not shown).

### 2.3.2 Sex ratio

Fish were separated into four distinct sex categories: female, male, hermaphrodite, and unknown (Stage 0). Both species were predominately females. We sampled a total of 2204 females, 1495 males, 9 hermaphrodites, and 196 unknowns for *E. stoutii* and 194 females, 88 males, and 21 unknowns for *E. deani*. Figure 5 shows a similar pattern in the distribution of reproductive stages across all seasons for *E. stoutii*. Approximately 56% of the *E. stoutii* population was female when including unknowns and simultaneous hermaphrodites or 1.47:1 when accounting for females-to-males only. The *E. deani* population was 64% female or a ratio of 2.20:1 when accounting for females-to-males only.
Sex ratios were subdivided into 25 mm length bins to assess ontogenetic changes in sex ratios (Fig. 6). Although both species were heavily dominated by females, they reached large ratios at opposite size extremes. The Pacific hagfish was heavily dominated by females at lengths less than 300 mm reaching a maximum of 50:1, but became slightly male dominated above 500 mm (Fig. 6). Males were not found under 225 mm, while 17 females were found between 175-225 mm (Fig. 6). Similarly no females were found larger than 650 mm, while 3 males were found from 650-775 mm. The Black hagfish maintained a 3:1 sex ratio in sizes less than 350 mm and declined to 0.9:1 around 400 mm only to jump to 23:1 at lengths greater than 500 mm. No males were found smaller than 275 mm nor greater than 525 mm. Only one female was found smaller than 275 mm while 25 females were found between 525-600 mm.

2.3.3 Fecundity
The average fecundity for *E. stoutii* and *E. deani* Stages 3+ was 26.75 ±16.58 (SD) and 32.57± 20.57 (SD), respectively. The average fecundity for Stages 1 and 2 was 150+ and appeared to indicate a maintained standing stock of eggs in all females throughout their reproductive lifespan; as a result these stages were excluded from the average fecundity. The highest fecundity was found in Stages 3 and 4 and then significantly declined through Stage 7 for both Pacific (*p* < 0.01, *r²* = 0.18) and Black hagfish (*p* < 0.01, *r²* = 0.37) (Table 4). Through macroscopic observations we noticed some of the maturing eggs undergo atresia resulting in the decreasing fecundity with increasing reproductive stage. This suggests hagfish may reduce reproductive capabilities as a result of changes in energetics.
Results of two-sample t-tests comparing mean fecundity between species and reproductive stages were variable in significance (Table 4). The Black hagfish had overall significantly (p<0.01) higher mean fecundity than Pacific hagfish. However, only reproductive Stages 3 and 5, were significantly different (p<0.05) between species with a higher mean fecundity in E. deani.

Post-ovulatotory follicles (POFs) were counted in Stage 8 females as a proxy for post-release fecundity. However, in only 17 hagfish of 94 Pacific Stage 8s and 2 hagfish of 18 Black Stage 8s were POFs able to be counted. Furthermore, the time for POFs to degenerate in hagfish is unknown, therefore, the accuracy in our measurements of Stage 8 fecundity in Table 4 is limited.

Pacific and Black hagfish had variable trends in regards to egg length and fecundity compared to total length and egg length (Fig. 7). Pacific hagfish fecundity increased significantly ($r^2=0.085$, p<0.01) with total length while the same relationship was not significant in Black hagfish ($r^2=0.002$, p>0.05) (Fig. 7A). The lack of relationship seen in Black hagfish could be the result of a low sample size across a large depth range (100-760 m) and sampling across multiple Pacific Fishery Management Areas (PFMA 123, 124, 125). Additionally, Pacific hagfish egg length increased significantly with total length ($r^2=0.027$, p<0.01) whereas the same relationship was not significant in Black hagfish ($r^2=0.017$, p>0.05) (Fig. 7B). A significant negative trend was observed for both E. stoutii ($r^2=0.20$, p<0.01) and E. deani ($r^2=0.31$, p<0.01) when comparing fecundity to egg length (Fig. 7C).

We also found significant differences in the interaction of reproductive stages and species (p<0.01). For E. stoutii Stages 2 and 3, and Stages 4 and 5 were significantly
(p<0.01) different with the earlier stages having a greater mean fecundity. There were no other significant (p>0.05) differences between stages in progression (i.e. Stages 3 and 4, Stages 5 and 6, Stages 6 and 7). In the case of *E. deani* Stages 3 and 4 and Stages 4 and 5 were significantly (p<0.05) different with the earlier stage having a greater mean fecundity. Stages 5 and 6 and Stages 6 and 7 were not significantly different (p>0.05) from each other.

### 2.3.4 Development curves

Results of estimating TL$_{50}$, the total length at which 50% of the population begins development of mature ovaries or testes, suggests males begin development at a later stage than females for both species. *E. stoutii* and *E. deani* females (225.79 mm, 298.71 mm, respectively) developed at a smaller size than males (325.97 mm, 333.00 mm, respectively) (Fig. 8A and 8B). Figure 8C suggests that at TL$_{50}$ half the population of Stage 0 hagfish have commenced sex differentiation into females or males. However, it is also possible that Stage 0 hagfish do not differentiate into either males or females and are instead sterile (Schreiner 1955).

### 2.4 Discussion

The reproductive and biological traits found in this study largely reflect the results from previous studies of Pacific and Black hagfish fisheries. Weight-length relationships show sexual dimorphism in both Pacific and Black hagfish across all known populations (this study, Barss 1993, Johnson 1994). Black hagfish are significantly larger than Pacific hagfish; however, hagfish of differing sexes across species are of similar size, which in this study is the result of inter- and intra-species morphological differences rather than size selectivity of the traps. Females in both species commence maturation at
significantly smaller sizes than the males. Furthermore, the sex ratio of Pacific hagfish decreases from heavily female to slightly male dominated, thus, this study corroborates the suggestion that Pacific hagfish are juvenile protogynous hermaphrodites (Gorbman 1990, Johnson 1994). In contrast, male maturation size and increasing female-to-male sex ratio for Black hagfish suggest that either this species is dioecious, dioecious with juvenile hermaphroditism, or protandrous hermaphroditic with delayed male maturation. Dioecious appears to be the most probable, however, more histological studies are required in Black hagfish to determine whether juvenile hermaphroditism exists. Both species have a very low fecundity ranging from 14 – 33 eggs per female. However, we are still unable to identify any seasonal or annual spawning patterns in Pacific hagfish in British Columbia (all Black hagfish were collected in the summer) (see below).

Additionally, 90.3% of the Pacific hagfish female catch was 8-12 years old and mature, suggesting that the current BC Pacific hagfish fishery is targeting a substantial number of large, mature female hagfish.

2.4.1 Review of reproductive terminology and sex determination in *E. stoutii* and *E. deani*

Previous hagfish reproductive studies lacked detailed reproductive terminology, therefore, we decided to provide a small summary with up-to-date reproductive fisheries terminology (following Lowerre-Barbieri et al. 2011). Reproductive studies suggest Pacific and Black hagfish are an iteroparous, *k*-selected species with an asynchronous spawning cycle (Gorbman 1990, Barss 1993, Johnson 1994, Leask and Beamish 1999, Benson et al. 2001). Moreover, the maintained standing stock of oocytes through ovulation and recrudescence observed in this study and others suggests that both species are batch spawners with indeterminate fecundity (Gorbman 1990, Barss 1993, Johnson
Previous histology suggests that Pacific hagfish are juvenile protogynous hermaphrodites wherein an unknown epigenetic factor triggers the female to male change prior to the start of maturity (Gorbman 1990). Additionally, we found a low incidence (0.03 – 0.23% of the population) of simultaneous hermaphroditism in mature Pacific hagfish agreeing with previous studies (Barss 1993, Johnson 1994). Comparatively, a lack of histological data for Black hagfish prevents any determination of sex prior to maturity, however, based on macroscopic observations Johnson (1994) suggested that protogynous hermaphroditism is unlikely to occur in *E. deani*, which the observations and sex ratios in this study corroborate. The increasing prevalence of females at larger sizes suggest that *E. deani* is a protandrous hermaphrodite, however, visual observations and the pattern in sex ratios are typical of many hagfish species suggesting instead a dioecious species (Gorbman and Dickhoff 1978). Whether the dioecious Black hagfish has a juvenile bisexual or hermaphroditic stage is unknown. Furthermore, simultaneous hermaphroditism in mature Black hagfish was found to occur in 0.24% of the population in California (Johnson 1994).

### 2.4.2 Length-weight relationships across fisheries

Despite increased interest in hagfish fisheries, there is a dearth of length/weight data for both *E. stoutii* and *E. deani*. This study targeted areas that have been previously surveyed in earlier hagfish fisheries such as Kirby Point (PFMA 23-7) and Amphitrite Point (PFMA 123-5). The mean length for the Kirby Point 1989-1992 Pacific hagfish fishery was larger (415 mm ± 10.1 SE) than both the 2000-2001 fishery (401 mm ± 2.24 SE) and the 2013-2016 fishery (387 mm ± 3.19 SE) (Leask and Beamish 1999, Benson et al. 2001). Furthermore, the length range at Kirby Point has decreased since the 1989-
1992 fishery from 160-720 mm to 208-597 mm in the current fishery (Leask and Beamish 1999, Benson et al. 2001). Similar traps were used across fisheries at Kirby Point, suggesting that selectivity should be similar, but perhaps larger and smaller fish have migrated to a different location. However, we would not expect fishing to cause such narrowing given the lack of fishing for 10-15 year periods between fisheries. At Amphitrite Point the mean length for Pacific hagfish has decreased slightly from 438 mm ± 5.39 SE in 1989-1992 to 431 mm ± 2.56 SE in the 2013-2016 fishery (Leask and Beamish 1999). However, it should be noted that the mean length for the total population of Pacific hagfish in the current fishery (428 mm ± 1.23 SE) is only slightly larger than location-specific means from the 1989-1992 and 2000-2001 fisheries suggesting consistency in sizes across the subpopulations and perhaps movement between them.

In comparison to other fisheries, *E. stoutii* mean length in BC was larger than that found in California (336 mm ± 19.4 SE) and Oregon (396 mm ± 3.52 SE) (Barss 1993, Johnson 1994). Although the minimum size captured was identical between BC and California at 107 mm, the minimum found in Oregon was larger at 200 mm. Moreover the maxima for Oregon (670 mm) and California (508 mm) were much smaller than that found in the BC fisheries (754 mm). The differences in minima are the result of variable sized escape holes in each trap between the fisheries (Barss 1993, Johnson 1994). The differences in maxima may be the result of a multitude of variables such as food resources, location, trap soak time, and depth.

The mean length for *E. deani* (432 mm ± 4.06 SE, 256-598 mm) in the BC fishery was much larger than that of the Oregon fishery (345 mm ± 4.19 SE, 200-520 mm) or California fishery (349 mm ± 20.6 SE, 213-543 mm) (Barss 1993, Johnson 1994). The
differences in mean length can be explained by differing size-selective gear between studies. For instance Johnson (1994) used traps with 7 mm holes and Barss (1993) used traps with 8 mm holes, while most of the traps in this study had 16 mm holes. Therefore, our study may have missed a substantial portion of smaller hagfish due to the larger escape holes or perhaps we missed smaller Black hagfish habitats. Black hagfish were only caught as bycatch in the previous two BC fisheries resulting in no substantial data, therefore, we are unable to make any direct comparisons to the current fishery.

Overall the mean length for Pacific hagfish (428 mm ± 1.23 SE) was slightly smaller than the mean length for Black hagfish (432 mm ± 4.06 SE), however, intraspecific differences were variable. Male Pacific hagfish were significantly larger in weight-at-length than female Pacific hagfish. This intraspecific variation could be the result of differing energy usage with females utilizing more energy for maintaining a standing stock of oocytes or perhaps it is a consequence of protogynous hermaphroditism in *E. stoutii*. In contrast, female Black hagfish were significantly larger in weight-at-length than male Black hagfish. The sexual dimorphism in Black hagfish was much more extreme than in Pacific hagfish as observed in Figure 3, thus, perhaps the observed intraspecific variation in Black hagfish is the result of an evolutionary shift in deep-sea fish towards larger females, which provide males with a better chance to fertilize a female via random occurrence therefore increasing reproductive success of the population (Stein and Pearcy 1982, Warner 1984). Interspecific variation can be attributed to differing growth rates between species or differing metabolic rates between a shallower and deeper species (Pacific: 60-220 m, Black: 140-750 m). Furthermore, interspecific variation in weight-at-length between *E. stoutii* and *E. deani* appears to be consistent
across North American populations, therefore, it is likely the result of habitat or metabolic differences (Barss 1993, Johnson 1994).

2.4.3 Sex ratio and development curves

Both hagfish species had strongly female-dominanted sex ratios. However, in both species sex ratios changed across length-classes, but in opposite directions. Pacific hagfish exhibited a large ratio of 50:1 females-to-males at the smallest sizes (250 mm), however, the average ratio became 1.6:1 after 300 mm. This female skewed sex ratio persisted further until 500 mm + where the ratio switched to male dominated ratios. A similar progression in sex ratio in Pacific hagfish was noted in California (Johnson 1994). Gorbman (1990), after histological examination, suggested that *E. stoutii* were protogynous hermaphrodites in their juvenile stage and commenced male differentiation at 280 mm in length; a result that has been corroborated by other studies (Johnson 1994, Leask and Beamish 1999, Benson et al. 2001). However, it has also been suggested that *E. stoutii* males and females have asynchronous growth rates rather than exhibiting juvenile protogynous hermaphroditism (Martini and Beulig 2013). Our results showed that the TL50, or length at which development began, for males and females were significantly different with males starting development at larger lengths further corroborating juvenile protogynous hermaphroditism instead of sex-based growth differences. Additionally, the progression towards male-dominated sex ratios at larger sizes may also be the result of reduced growth rates as large females (642 mm) were still found within our samples albeit at a lower abundance. In conclusion, our results strongly corroborate those of Gorbman (1990) suggesting that *E. stoutii* are protogynous
hermaphrodites in their juvenile stage. However, much work is still needed, including the identification of potential environmental or social cues for differentiation.

In contrast, Black hagfish were female-dominated at both size extremes with a high ratio of 23:1 (female-to-male) at 500+ mm, but reached a 0.9:1 ratio around 400 mm. This is likely a typical *E. deani* pattern, however, the extreme increase in sex ratio at larger sizes is likely the result of a low sample size (n=303) and location-specific fishing pressure (Gorbman and Dickhoff 1978, Johnson 1994). Due to the lack of histological studies, we are still unable to determine whether *E. deani* are dioecious, dioecious with a juvenile hermaphroditic stage, or protandrous hermaphrodites. However, the development curves showed a significant difference in TL50 between males and females, suggesting either delayed male maturation or protogynous hermaphroditism. Results of previous studies suggest *E. deani* is highly unlikely to be protogynous, thus, it is likely *E. deani* is dioecious with a delayed male maturation or protandrous hermaphroditic (Barss 1993, Johnson 1994). A full histological analyses is necessary to properly identify their reproductive system.

We also found simultaneous hermaphroditic *E. stoutii* at larger sizes. Mature simultaneous hermaphrodites are defined as hagfish that maintained mature egg production in the anterior 2/3\(^{rd}\) of the gonadal fold while simultaneously growing mature testes in the posterior 1/3\(^{rd}\) of the gonadal fold. Overall we found nine adult simultaneous hermaphrodites, or a total incidence of 0.23% ranging in size from 388 mm to 634 mm. A similar low incidence (0.20%) of mature simultaneous hermaphrodites was found in the Oregon fishery while a much lower (0.03%) incidence of mature simultaneous hermaphrodites was found in California (Barss 1993, Johnson 1994). The reported
incidence of hermaphroditism in larger hagfish is also low in other species such as *Eptatretus cirratus* (0.25%), *Eptatretus burgeri* (<0.1%), and *Eptatretus deani* (0.24%) (Patzner 1977, Johnson 1994, Martini and Beulig 2013).

The Stage 0 development curves suggest that undifferentiated Black hagfish are significantly larger than Pacific hagfish. For both species the TL\textsubscript{50} of Stage 0 is slightly less than that of the TL\textsubscript{50} of females suggesting that most Stage 0 hagfish are likely females that were unidentified macroscopically. Furthermore, Stage 0 hagfish at larger sizes, such as those larger than the TL\textsubscript{50} of females and males, may be sterile or have a longer-than-average delayed development (Schreiner 1955). From the length data for Stage 0 hagfish in both species, we can assume that any outliers, generally 375 mm +, are likely sterile. Moreover, the lack of Stage 0 hagfish greater than 460 mm in Pacific hagfish and 398 mm in Black hagfish suggests sterile hagfish suffer an earlier natural mortality.

### 2.4.4 Comparing fecundity between species and fisheries

The peak fecundity of Pacific and Black hagfish is best represented by Stage 5+ females rather than over the entire female population due to the significant decline in fecundity with increasing reproductive stages. The low fecundity of Pacific and Black hagfish found in British Columbia populations is consistent with that of other populations (Gorbman 1990, Barss 1993, Johnson 1994). Furthermore, low fecundity is common amongst deep-sea species, which tend to be on the far K-selected end of the life history spectrum (Koslow et al. 2000).

Consistent with previous findings, correlations between fecundity and total length were weak, but significantly positive for *E. stoutii* (Johnson 1994). The correlation
increased with reproductive stage indicating that total length has some effect on fecundity. This significantly positive, yet, weak correlation can be explained by the cyclical nature of the hagfish reproductive cycle, and the usage of the reproductive stages in this study. Unlike male hagfish, which mature linearly with growth, female hagfish go through multiple reproductive ‘maturity’ cycles (Stages 1-8). Furthermore, the variability observed in both number of eggs and egg length against total length is directly the result of female hagfish progressing through reproductive Stages 1-8 at different lengths in time, and in essence different ages. Thus, the variation observed in female length with reproductive stage, fecundity, and egg length can all be primarily explained by the cyclical nature of their reproductive biology. However, *E. deani* total length did not significantly correlate with fecundity. This is likely the result of a low sample size and high variability leading to non-significant trends. We assume the large variation observed in *E. deani* populations is due to similar reasons as the observed variation in *E. stoutii*; a result of the cyclical nature of their reproductive biology.

In our study both species had statistically similar ($p > 0.05$) mean egg lengths in females Stage 5+, 26 mm ± 3.2 SD for Pacific and 27 mm ± 3.5 SD for Black hagfish. Furthermore, mean egg length for Pacific hagfish in this study was greater than that in California (24 mm for eggs >15+ mm) or Oregon (14.3 mm for eggs >5+ mm) (Barss 1993, Johnson 1994). These results suggest that in our study we sampled a substantial number of females further along the reproductive cycle than in previous studies, but may also be the result of differences in sample sizes between seasons. In contrast, mean egg length for Black hagfish was between that of California (34 mm for 15+ mm eggs) and Oregon (21 mm for 5+ mm eggs) (Barss 1993, Johnson 1994). This difference could be
the result of seasonal differences, or perhaps depth as Johnson (1994) caught most of his Black hagfish at a greater depth (750-1000 m) than our study (100-760 m in BC) and Barss (1993) (366-456 m) and found substantially larger eggs.

For both species, fecundity decreased from ~200 eggs in Stage 1 to a mean of 18 and 20 in Stage 7 for *E. stoutii* and *E. deani*, respectively. The decrease in the number of eggs with increasing egg length is likely due to the energetic cost to maintain yolk production in larger eggs (Gorbman 1990, Johnson 1994). The mean fecundity of females with mature, developing eggs for *E. stoutii* was less than that of *E. deani*, which may be the result of Pacific hagfish having smaller body cavities than Black hagfish (Johnson 1994). Additionally, in both species we found that the trade-off between egg size and egg number in larger females is not substantial. However, recent studies have shown that in many fish species larger females may disproportionately contribute to both egg production and reproductive success (Law 2000, Murawski et al. 2001, Berkeley et al. 2004). Furthermore, these large females may contribute more to self-recruitment or local replenishment of the population, which may be the result of age-related differences in location and time of spawning or that older fish may produce higher quality larvae (Berkeley et al. 2004, Beldade et al. 2012).

### 2.4.5 Age estimation

There is currently only one study that estimates age and growth of *E. stoutii*. Nakamura (1994) suggested that 180-200 mm long hagfish are 4-8 years old, and 450-500+ mm hagfish are 15-20+ years old. He also estimated that the age at maturity for females was 8-12 years old at a size of 325 mm (Nakamura 1994). If we apply this
estimate to the BC female population (n=2204) then 90.3% of the female population was mature.

2.4.6 Seasonal distributions

Seasonal shifts in mature hagfish can be used to identify possible spawning periods. In the case of *E. stoutii*, in the summer we found a large abundance of mature males and females as well as a large abundance of immature females. Similar patterns are observed across seasons but at a lesser abundance. In the California population *E. stoutii* spawning is related to seasonal changes with spawning occurring in the winter (Stephens 1995). However, no similar patterns have been observed in the British Columbia population (Benson et al. 2001). This could be attributed to differences in population dynamics, however, previous California studies also found no spawning periodicity in relation to seasons (Johnson 1994). The only species with a confirmed seasonal spawning cycle is *E. burgeri* (Fernholm 1975, Patzner 1977, 1978). Although seasonal periodicity has not been observed directly in Pacific hagfish, recent studies have shown a correlation between immunoreactive (ir) gonadotropin releasing hormone (GnRH) and gonadal reproductive stages (Powell et al. 2005, Nozaki 2013). Thus, it is possible that some *Eptatretus* spp have seasonal spawning cycles, but based on our data it is likely that *E. stoutii* and *E. deani* are asynchronous species spawning throughout the year.

2.4.7 Conclusion

The reproductive characteristics of *E. stoutii* and *E. deani* create a variety of concerns for the implementation of a sustainable hagfish fishery. These traits includes 1) a low fecundity and late maturation, 2) egg production in asynchronous species can take 2-3 years (Patzner 1978), and in the case of *E. burgeri* required a minimum of 7 months
(Ota et al. 2007), 3) the large abundance of immature females, and undifferentiated or sterile hagfish, and 4) older, larger females may disproportionately contribute to population reproductive success and recruitment. Furthermore, other reproductive features such as skipped spawning, fisheries-induced evolution, and the trade-off between survival, reproduction and growth should be accounted for when estimating the spawning stock biomass in fisheries (Lowerre-Barbieri et al. 2011). Each of these can impact fecundity and spawning seasons, sex allocation in hermaphrodites and onset of sexual maturity, and a species’ reproductive lifespan (Lowerre-Barbieri et al. 2011).

It remains necessary to gather more detailed seasonal data for both species of hagfish in BC to identify any seasonal migrations or spawning cycles. We recommend that future research targets ir-GrNH cycles in *E. stoutii* and *E. deani* alongside reproductive stages in order to identify seasonal or annual spawning cycles. Furthermore, a methodology to age hagfish is still required. Attempts at utilizing micro-statoconia to age hagfish failed (Lee et al. 2007). Instead, we recommend using mark-and-recapture methods similar to those of Nakamura (1994) to age and eventually estimate growth rates.

Hagfish fisheries continue to be of interest around the world, however, it is necessary to prevent the population collapses observed in historical hagfish fisheries (Gorbman et al. 1990). So far it appears the experimental fishery in British Columbia has targeted mature hagfish. Given our results, we recommend that escape holes are adjusted to reduce capture of undifferentiated and immature hagfish to allow them to mature. More seasonal biological information is required from the ongoing experimental fishery.
This information can be obtained by continuing to survey the fishery while implementing the above suggested improvements.
Bibliography


Patzner, R.A. 1978. Cyclical changes in the ovary of the hagfish Eptatretus burgeri


Figures

Figure 1. A) A quadratic curve (LogW=a+bL+cL^2) of log weight versus total length for *E. stoutii*; a = -6.530e^{-02}, b = 1.610e^{-02}, c = -1.122e^{-05}
B) A quadratic curve (LogW=a+bL+cL^2) of log weight versus total length for *E. deani*; a = -1.765e^{-01}, b = 1.784e^{-02}, c = -1.293e^{-05}
Figure 2 Seasonal comparison of length-weight relationships for *E. stoutii*. In larger hagfish the hagfish are heaviest in the winter and lightest in the spring. In smaller hagfish the hagfish are heaviest in summer and lightest in fall.

LogW = a + bL + cL^2;
- Summer: a = -7.145e^{-02}, b = 1.628e^{-02}, c = -1.153e^{-05}, n=2437, r^2=0.92
- Fall: a = -9.272e^{-02}, b = 1.539e^{-02}, c = -9.904e^{-06}, n=497, r^2=0.92
- Winter: a = -5.725e^{-02}, b = 1.577e^{-02}, c = -1.051e^{-05}, n=771, r^2=0.92
- Spring: a = -5.984e^{-02}, b = 1.685e^{-02}, c = -1.291e^{-05}, n=199, r^2=0.92

F(7, 3896) = 20.57; p<0.01, seasons are significantly different.
Figure 3  A) *Eptatretus stoutii* comparison of male and female length-weight curves using the standard fisheries equation $W=aL^b$. The curves are significantly different with males heavier than females.

Female: $W = 7.0602 \times 10^{-6} L^{2.741}$
Male: $W = 2.375 \times 10^{-5} L^{2.545}$

B) *Eptatretus deani* comparison of male and female length-weight curves using the standard fisheries equation $W=aL^b$. The curves are significantly different with females heavier than males.

Female: $W = 6.247 \times 10^{-6} L^{2.813}$
Male: $W = 2.054 \times 10^{-5} L^{2.622}$
Figure 4 A) Total length compared to reproductive stages for *E. stoutii*. B) Total length compared to reproductive stages for *E. deani*. 
Figure 5 A seasonal comparison of reproductive stages for *E. stoutii*. A similar pattern is observed across all seasons.
Figure 6  A) *Eptatretus stoutii* sex ratios (female:male) as a function of total length sorted into 25 mm total length bins. The mean sex ratio was 1.47 with a range of 50:1 at 250-274 mm to 0.7:1 at 500-524 mm.

B) *Eptatretus deani* sex ratios (female:male) as a function of total length sorted into 25 mm total length bins. The mean sex ratio was 2.20 with a range of 0.9:1 at 400-424 mm to 23:1 at 500-524 mm.
Figure 7 A) Fecundity as a function of total length. There is a significant relationship for *E. stoutii* (left), but no significant relationship for *E. deani* (right).

B) Egg length as a function of total length. There is a significant increase in egg length with total length for *E. stoutii* (left), but no significant relationship for *E. deani* (right).

C) Fecundity as a function of egg length. Fecundity decreased significantly with increasing egg length for both species.
Figure 8 Sexual development curves for *E. stoutii* (left) and *E. deani* (right). The data were sorted into 50 mm bins. Each curve used a variation of the three-parameter logistic equation: \( \text{Percent} = 100 / (1 + (\text{TL}_{50} / X)^b) \).

A) Female development curve. Percentage for females was determined as:
(Female/(Female+Unknown)). *E. stoutii*: \( \text{TL}_{50} = 225.79, b = 4.10; E. deani: \text{TL}_{50} = 298.71, b = 8.53 \)

B) Male development curve. Percentage for males was determined as:
(Male/(Male+Unknown)). *E. stoutii*: \( \text{TL}_{50} = 325.97, b = 14.04; E. deani: \text{TL}_{50} = 333.00, b = 12.82 \)

C) Unknown development curve. Percentage for unknowns was determined as:
(Unknown/(Female+Male+Unknown)). *E. stoutii*: \( \text{TL}_{50} = 222.61, b = -4.29; E. deani: \text{TL}_{50} = 288.79, b = -9.41 \)
Tables

Table 1. Stages of sexual differentiation for both *E. stoutii* and *E. deani*. The methodology was adapted from Gorbman (1990) and Martini and Beulig (2013). The descriptions are based on macroscopic observations. The female stages are cyclical whereas the male stages are linear over their lifetime.

<table>
<thead>
<tr>
<th>Reproductive Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unknowns</strong></td>
<td></td>
</tr>
<tr>
<td>Stage 0</td>
<td>No identifiable gonads; undifferentiated</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>Eggs &lt;1 mm</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Eggs 1.01-3 mm</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Eggs 3.01 - 7 mm</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Eggs 7.01 -15 mm</td>
</tr>
<tr>
<td>Stage 5</td>
<td>Eggs 15.01 - 23 mm</td>
</tr>
<tr>
<td>Stage 6</td>
<td>Eggs 23.01 - 31 mm</td>
</tr>
<tr>
<td>Stage 7</td>
<td>Eggs 31.01+ mm</td>
</tr>
<tr>
<td>Stage 8</td>
<td>Large, flaccid postovulatory follicles indicating recent spawning activity</td>
</tr>
<tr>
<td><strong>Hermaphrodites</strong></td>
<td></td>
</tr>
<tr>
<td>Stage 9</td>
<td>Mature testes and ovaries simultaneously present</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
</tr>
<tr>
<td>Stage -1</td>
<td>Testicular band present</td>
</tr>
<tr>
<td>Stage -2</td>
<td>Small testicular follicles containing fluid</td>
</tr>
<tr>
<td>Stage -3</td>
<td>Enlarged testicular follicles, cloacal gland &lt;12 mm in length</td>
</tr>
<tr>
<td>Stage -4</td>
<td>Distended testicular follicles, cloacal gland 12+ mm in length</td>
</tr>
</tbody>
</table>

Table 2. Mean length (mm), standard deviation (SD), and range for both *E. stoutii* and *E. deani*. No simultaneous hermaphrodites were found in *E. deani* samples.

<table>
<thead>
<tr>
<th></th>
<th><em>E. stoutii</em></th>
<th>E. deani</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>428</td>
<td>76.7</td>
<td>107-754</td>
</tr>
<tr>
<td>452</td>
<td>62.8</td>
<td>236-754</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>423</td>
<td>72.3</td>
<td>174-642</td>
</tr>
<tr>
<td><strong>Unknowns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>288</td>
<td>59.3</td>
<td>107-460</td>
</tr>
<tr>
<td><strong>Hermaphrodites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>535</td>
<td>78.8</td>
<td>388-634</td>
</tr>
</tbody>
</table>
Table 3. Post-hoc Tukey HSD comparison test comparing mean lengths against sex (F= Female (Stages 1 to 8), M= Male (Stages -1 to -4), U= Unknown (Stage 0), H= Hermaphrodite (Stage 9)) within species (Pacific, Black) and the interaction between sexes and species. Means with the same letter in superscript are not significantly different.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Mean Length (mm)</th>
<th>Species:Gender</th>
<th>Mean Length (mm)</th>
<th>Gender</th>
<th>Mean Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>536\textsuperscript{a}</td>
<td>Pacific:H</td>
<td>536\textsuperscript{a}</td>
<td>F</td>
<td>451\textsuperscript{a}</td>
</tr>
<tr>
<td>M</td>
<td>452\textsuperscript{b}</td>
<td>Pacific:M</td>
<td>452\textsuperscript{b}</td>
<td>M</td>
<td>417\textsuperscript{b}</td>
</tr>
<tr>
<td>F</td>
<td>423\textsuperscript{c}</td>
<td>Black:F</td>
<td>451\textsuperscript{b}</td>
<td>U</td>
<td>323\textsuperscript{c}</td>
</tr>
<tr>
<td>U</td>
<td>288\textsuperscript{d}</td>
<td>Pacific:F</td>
<td>423\textsuperscript{c}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black:M</td>
<td>417\textsuperscript{c}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black:U</td>
<td>323\textsuperscript{d}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pacific:U</td>
<td>288\textsuperscript{d}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Average fecundity ± standard deviation for all females 3+ of each species and for reproductive stages with less than 150 eggs (3-8). *E. deani* average fecundity was always greater than *E. stoutii* except for Stage 8, however it was not always significant.

<table>
<thead>
<tr>
<th>Reproductive Stage</th>
<th><em>E. stoutii</em></th>
<th><em>E. Deani</em></th>
<th>p</th>
<th>t</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3+)</td>
<td>26.75 ± 16.58</td>
<td>&lt; 32.57 ± 20.57</td>
<td>0.00627</td>
<td>-2.7827</td>
<td>119.14</td>
</tr>
<tr>
<td>Stage 3</td>
<td>38.32 ± 24.65</td>
<td>&lt; 68.27 ± 38.12</td>
<td>0.0269</td>
<td>-2.5637</td>
<td>10.672</td>
</tr>
<tr>
<td>Stage 4</td>
<td>33.68 ± 17.78</td>
<td>&lt; 36.39 ± 14.24</td>
<td>0.305</td>
<td>-1.0373</td>
<td>51.280</td>
</tr>
<tr>
<td>Stage 5</td>
<td>19.60 ± 6.56</td>
<td>&lt; 24.45 ± 6.91</td>
<td>0.000639</td>
<td>-3.7462</td>
<td>35.656</td>
</tr>
<tr>
<td>Stage 6</td>
<td>20.30 ± 7.56</td>
<td>&lt; 22.05 ± 6.58</td>
<td>0.261</td>
<td>-1.1456</td>
<td>28.921</td>
</tr>
<tr>
<td>Stage 7</td>
<td>17.35 ± 5.00</td>
<td>&lt; 20.75 ± 2.06</td>
<td>0.0532</td>
<td>-2.1346</td>
<td>12.543</td>
</tr>
<tr>
<td>Stage 8</td>
<td>19.47 ± 3.97</td>
<td>&gt; 14.00 ± 5.66</td>
<td>0.3926</td>
<td>1.3297</td>
<td>1.1119</td>
</tr>
</tbody>
</table>