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EPIDEMIOLOGY OF PUFFY SNOUT SYNDROME
IN TUNA

BY
TAYLOR M. VOORHEES

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
FISHERIES, ANIMAL AND VETERINARY SCIENCE

UNIVERSITY OF RHODE ISLAND

2015

MASTER OF SCIENCE THESIS
OF
TAYLOR M. VOORHEES

APPROVED:

Thesis Committee: Marta Gomez-Chiarri
Susanne Menden-Deuer

Major Professor: Terence M. Bradley

Nasser H. Zawia
DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
2015

ABSTRACT

The domestication of tuna species has proven to be one of the most challenging endeavours in aquaculture. Among the issues yet unresolved is the occurrence of ‘puffy snout syndrome,’ a condition in which tumour-like growths form on the head. Ultimately, vision and feeding are impaired and long-term afflictions typically result in mortality. Though few mentions of puffy snout exist in the literature, evidence suggests that it is not uncommon among facilities that hold tunas in captivity. The specific aims of this study were to: a) describe pathological features of puffy snout, and b) investigate its etiology through the evaluation of conditions and protocols at facilities rearing/holding tuna.

To describe pathological features of puffy snout, clinical signs were detailed by observing captive tunas in a land-based holding system, and examination of evidence of infection by parasites, bacteria, and viral agents was conducted on tissues collected from fish with and without puffy snout. Histological examination of tissue from normal and affected fish was also conducted. To investigate etiology, a survey was developed and sent electronically to 28 tuna-holding facilities globally. The survey inquired about the prevalence of puffy snout and the husbandry conditions and protocols employed (e.g., biological characteristics, capture and transport procedures, holding system design and water quality, feeding regime). These data were compared across facilities in an attempt to couple puffy snout prevalence with holding conditions and/or protocols.

Clinical signs of puffy snout included occlusion of the eyes and mouth, followed by changes to swimming and feeding behaviours. Parasitology, bacteriology, and

virology examination all indicated no commonly-found pathological agents were responsible for inducing the condition. Histology showed that puffy snout is largely characterised by the apparent degeneration of muscle tissue with the replacement of a loose collagenous fibrosis and an undetermined fluid filling the interstitial space in tissues anterior to the eye. In dorsal musculature, collagenous growth may occur in the epidermal or hypodermal regions.

Based on survey data, the capture and transport process and feeding regime were unrelated to development of puffy snout. However, certain biological (e.g., fish size) and holding system (e.g., tank/pen size) parameters showed weak but non-dismissible coupling with puffy snout prevalence. Survey data and additional personal communication with field researchers confirmed that puffy snout in tuna is solely a captivity-related condition.

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The completion of this work would not have been possible without the personal and professional support and assistance of many. My gratitude is humbly extended to each of you: Dr. Terry Bradley, for the opportunity to learn more than I could have ever foreseen, your belief in my ability to do so, and your dedicated guidance through the process; Dr. Marta Gomez-Chiarri and Dr. Susanne Menden-Deuer, for your input and expertise as members of my committee; those who returned a completed survey, for your invaluable data – I hope this work provides return on investment; Peter Mottur, for your commitment to and enthusiasm for the vision, and for your friendship – 'nuff respekt; Shaya Boymelgreen for making it possible; Chelsea Roy, Donald Bacoat, Ian Jaffe, and Tori Spence, for your assistance in so many aspects of the project; Katherine Favreau for keeping me hired; Paul Johnson for technical assistance; Ed Baker for keeping the water flowing; Maria Martin for keeping the floors polished and the chocolates.

To my family, born and acquired, thank you. Mom and Dad, for encouragement, love, sacrifice, and perspective. Jessie, asante kwa msukumo mimi kufuata ndoto yangu – ninakupenda. Sandy, for everything. The depth of my appreciation for all that you guys have done and continue to do for me could never be expressed. To my friends, thank you for being or pretending to be interested.

DEDICATION

This thesis is dedicated to Miss Amy Lynn. Thank you for always keeping me afloat.

PREFACE

The following thesis is written in manuscript format and adheres to the guidelines of the Graduate School of the University of Rhode Island. It is comprised of one manuscript, a literature review, and a bibliography; the manuscript is formatted in accordance with the guidelines set forth for publication in *Aquaculture*.

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MANUSCRIPT I

Prepared for submission to Aquaculture

EPIDEMIOLOGY OF PUFFY SNOUT SYNDROME IN TUNA

Taylor M. Voorhees and Terence M. Bradley

Department of Fisheries, Animal, and Veterinary Science

University of Rhode Island

Kingston, RI, USA

Corresponding author: Terence M. Bradley

Department of Fisheries, Animal, and Veterinary Science

University of Rhode Island

Kingston, RI, 02881, USA

Phone: +1-508-341-1673

E-mail: tbradley@uri.edu

1. INTRODUCTION

The domestication of tuna species has proven to be one of the most challenging endeavours in aquaculture. Among the issues yet unresolved is the occurrence of ‘puffy snout syndrome,’ a condition in which tumour-like growths form on the head region and, if the condition is severe, the eyes, mouth, and nares become occluded. Ultimately, vision and feeding are impaired and long-term affliction typically results in mortality. To date, there have been seven published reports of puffy snout (Tester, 1952; Nakamura, 1962, 1972; Dizon and Sharp, 1978; Queenth and Brill, 1983; Kaya et al., 1984; Benetti et al., 2009); none of which, however, offer detailed descriptions or characterisations of the pathology, nor submit comprehensive suggestions for its cause. All but one, Benetti et al., 2009, is in reference to its occurrence at the Kewalo Basin Research Facility on Oahu, Hawaii, USA. Anecdotal and personal evidence, however, suggest that puffy snout is hardly unique; rather, it is likely to afflict a variety of scombrid species at locations around the world.

1.1. *Prior Investigations of Puffy Snout*

In 1951, Albert Tester, working at the Kewalo Basin Research Facility, noticed a shift in feeding behaviour coupled with changes in skin colour and texture of a yellowfin tuna (*Thunnus albacares*) four months after its acquisition from the wild. Stating that the skin appeared “swollen,” Tester noted the inflammation interfered with the fish’s vision. The fish died less than three months later, and it appears no further investigation of the condition took place (Tester, 1952).

Nakamura (1962), also at the Kewalo lab, was the first to report the occurrence of puffy snout in skipjack tuna (*Katsuwonus pelamis*). He and others subsequently proposed that skipjack are more prone to the affliction than other tuna species (Dizon and Sharp, 1978; Nakamura, 1972; Queenth and Brill, 1983). In 1972, Nakamura termed the condition ‘puffy snout’ and gave the most detailed account to date:

“Tissues of the snout begin to swell and become edematous. The swelling spreads posteriorly until the fish is unable to close its jaws and the tissues around the eyes swell to give the appearance of sunken eyes. If left in this condition, the fish dies.”

It was suggested puffy snout is a “stress-related condition” (Kaya et al., 1984), potentially triggered by confinement (Dizon and Sharp, 1978; Nakamura, 1972). Notably, kawakawa (*Euthynnus affinus*) became afflicted with puffy snout when placed in an annular raceway less than one meter wide, but when transferred to a larger, 47 m³ (7 m diameter, 1.2 m deep) tank, the condition receded (Nakamura, 1972).

In 1983, Queenth and Brill reported that the occlusion of the eyes and covering of the teeth as a result of puffy snout prevents the fish from feeding, and may culminate in death by starvation. Kaya et al. (1984) also reported the condition to render fish unusable for experimental work and to be eventually fatal.

The most recent reference to puffy snout in published literature was by Benetti et al. (2009), where they reported that captive blackfin tuna (*Thunnus atlanticus*) had developed the malady. While investigation of the potential cause was limited, the report indicates that the fish did not eat while in captivity. Furthermore, for one 7 kg

mature tuna that died showing signs of puffy snout, they estimated that the fish had , travelled a distance of up to 2,000 km in the tank without feeding before perishing.

Outside of the Thunnini clade, puffy snout has been observed in mackerel species. Nakamura (1972) reported to have observed afflicted Pacific mackerel (*Scomber japonicas*) at Scripps Institution of Oceanography in La Jolla, California, USA, and afflicted Atlantic mackerel (*Scomber scombrus*) at The Plymouth Laboratory in Plymouth, England.

1.2. *Justification for Research*

Tuna species have been kept captive in both open-water and land-based units for several decades for research, commercial, and public display purposes. As concern mounts for the current and future health of many wild tuna populations (Miyake et al., 2010; FAO, 2011), there is increasing interest in ‘egg-to-plate’ tuna aquaculture, whereby captive broodstock produce progeny to be on-grown for eventual harvest. Operations in Japan, Australia, Panama, Indonesia, the United States, and several countries bordering the Mediterranean Sea have begun efforts to maintain and produce tunas in captivity. While many captivity-related hurdles have been addressed by these groups, there is a lack of evidence that any progress has been made toward understanding puffy snout. In addition to the published reports of puffy snout at Kewalo and at the University of Miami (and Nakamura’s observations at Scripps and Plymouth), there is confirmation via personal observation and personal communication with staff at other institutions that the malady is widespread. At the University of Rhode Island, Atlantic bluefin (*Thunnus thynnus*) and yellowfin held in

captivity developed puffy snout. Though confinement has been proposed as the specific stressor of fault, a short, informal investigation into the design and practices of tuna-holding facilities suggests that other factors may play a role. Additionally, no efforts into describing and characterising the anomalous tissues in detail have been published.

While the epidemiology and etiology of puffy snout are unknown, the clinical manifestations are clear: tumour-like growths interfere with at least vision and food ingestion. Furthermore, coping with these and other stressors may be deleterious to essential life functions. For example, the mounting of an immune response to other, potentially pathogenic threats may be compromised, and the physiological and behavioural processes that culminate in successful spawning might not transpire, as these are both characteristic effects of stress-coping in fish (Iwama, 1998; Harris and Bird, 2000; Schreck et al., 2001; Barton, 2002; Davis, 2006). Ultimately, the success of sustainable, 'egg-to-plate' tuna aquaculture depends on these physiological processes (among others) proceeding uninterrupted, as sub-par conditions often translate into a decrease or total absence of reproductive activity; this is especially true in the case of tunas, evidenced by the many hormonal induction efforts (Mylonas, 2003; Mylonas et al., 2007; de Metrio et al., 2010; Aranda et al., 2011; Rosenfield et al., 2012). Additionally, the unique physical and physiological capabilities of tuna species are of interest to researchers, yet, as stated by Kaya et al. (1984), puffy snout-afflicted fish are not representative of their congeners and of limited value in research investigations. Finally, the ability of public aquaria to showcase and share one of the oceans' most iconic fish may be compromised by the inability to keep them healthy.

Without a full understanding of the conditions under which puffy snout occurs and the effects it has on afflicted fish, long-term research efforts, progress in sustainable tuna aquaculture, and public display opportunities will be hindered.

The objectives of this study were to: 1) describe puffy snout at the morphological and cellular level and 2) investigate potential causes of the condition including tank design, water quality, capture and transport strategies, nutrition and other environmental and physiological factors.

2. MATERIAL AND METHODS

2.1. *Investigation of Pathology*

2.1.1. Animals Used for the Study

Ten fish were used to describe the pathology of puffy snout syndrome (Table 1). All fish were captured via rod and reel angling, as approved by the University of Rhode Island Institutional Care and Use Committee (URI IACUC) and permitted by the NOAA Atlantic Highly Migratory Species Division, from Atlantic Ocean waters between 30 and 175 nautical miles offshore from Rhode Island.

Eight of these fish were transported live from the fishing grounds to a land-based holding facility at the Blount Aquaculture Research Laboratory (BARL) on the University of Rhode Island's Narragansett Bay Campus, and held there until the time of their death or euthanasia. Transport and holding system infrastructure and fish care were intended to provide a suitable environment for the fish per previous investigations and published reports on holding tunas in captivity (e.g., Nakamura, 1972; Bourke et al., 1987; Brill, 1999, 2002; Farwell, 2001, 2003; Wexler et al., 2003; Sawada et. al, 2005; Margulies, et al., 2007; Benetti et al., 2009; Hutchinson et al., 2012) and personal communication with researchers (C. Farwell, M. Kelleher, G. Partridge). During the course of their residency in the BARL facility, all fish, with the exception of the false albacore (*Euthynnus alleteratus*), developed puffy snout syndrome. All eight fish, detailed below, serve as the captive group for this study.

Two Atlantic bluefin tuna, each exhibiting advanced-stage puffy snout, were euthanized on 7 February 2013 with tricaine methanesulfonate (MS-222) overdose and necropsied. They were captured in August 2012 and held at the BARL land-based facility until the time of sampling (Table 1).

Additionally, five yellowfin tuna captured between 7 October 2012 and 21 September 2013, exhibiting varying degrees of puffy snout severity were necropsied per Table 1. All five were held in the BARL land-based facility since collection.

In September 2012, twelve false albacore were captured and transported to the BARL land-based facility. Eleven were held until the time of their deaths and one was released on 31 October 2014. One mortality, which occurred on 22 April 2014, was necropsied at the time of death and is included in the captive group of this study (Table 1).

Two additional yellowfin were euthanized at sea immediately post-capture on 5 September 2014; these fish displayed no signs of puffy snout and were designated as “wild” fish. Euthanasia was completed by severing the spinal cord and exsanguination. The necropsy procedure, including the location of tissue samples, was identical to that employed for the captive group.

To limit the potential of pre-existing, capture-induced, or transport-induced health conditions, which could confound the later development of puffy snout, all fish were assessed for their general, grossly-observable health status at capture, during transport, and at the time of holding-tank-introduction. At capture, this clinical assessment included identifying the presence of wounds, abrasions, lesions, or otherwise compromising characteristics such as hook injury or line chafing. During transport,

normal swimming behaviour – defined as the absence of listing, wall contact, social aggression, and irregularity or difficulty in buoyancy regulation – was monitored. At the time of introduction to the holding tank, fish were once again examined and were evaluated for normal swimming. Fish condition and behaviour, including the commencement and continuation of feeding, was observed daily after introduction to the holding tank.

2.1.2. Transport and Holding Facility Infrastructure

Captive group fish were transported to the BARL facility by the fishing vessel in on-deck or in-deck tanks. All tanks were supplied with flowing, ambient seawater and oxygenated. The number of fish transported in a single trip was dependent upon several factors, including, but not limited to, size of individuals (target of <20 kg per fish), biomass density inside the transport tank (target of <5 kg/m³), and time at sea (target of fish in transport tank <24 hours before beginning return to facility).

The system used in the BARL facility was built in 2011-2012 for the purpose of maintaining tunas and conducting husbandry and breeding research. It consisted of a 74 m³ cylindrical fiberglass tank, supplied with seawater from Narragansett Bay. Prior to introduction to the tank, water was subject to two in-series filtration schemes. Influent water was filtered to 5 µm via sand filtration (Diamond Water Systems, Holyoke, MA, USA), and treated with ultraviolet light (UV) (Sunlight UV Systems, Equova Water Technologies, Warrendale, PA, USA). Influent water was maintained at 21 °C ±1 °C through the use of a commercial-scale boiler (Model 88 Series 1, Weil-McLain, Michigan City, IN, USA). Secondary filtration of influent water consisted of

a 1-hp centrifugal pump (Sea Flow®, Lifegard Aquatics, Cerritos, CA, USA) to provide sufficient head, three in-series 15 cm diameter, 50 cm height bag filters (X100, Filter Specialists, Inc., Michigan City, IN, USA) for removal of particulates to 1-25 μm , 30 mJ/cm^2 UV treatment (SMART HO ML300, Emperor Aquatics, Pottstown, PA, USA), degassing via a 30 cm diameter, 122 cm height de-gassing unit. Total influent water was introduced to the tank at 150-285 LPM. Tank water was also recirculated to maintain quality. Water was pumped from the tank via a 2-hp centrifugal pump (Sea Flow®, Lifegard Aquatics, Cerritos, CA, USA) and through two in-series 18 cm diameter, 78 cm height bag filters (XL234, Filter Specialists, Inc., Michigan City, IN, USA) to remove particulate matter. Dissolved organic matter and finer solids were removed via foam fractionation (RK75HS-HF, RK2, Escondido, CA, USA). Two in-parallel fluidised bed biological filtration units (RK75AC-FSF, RK2, Escondido, CA, USA) were used for nitrification followed by 30 mJ/cm^2 UV treatment (SafeGUARD CUP, Emperor Aquatics, Pottstown, PA, USA). Last, excess CO_2 and N_2 were removed by flowing the water through a vacuum-degassing unit (Water Management Technologies, Baton Rouge, LA, USA). Recirculated water flow totalled 265-386 LPM. Temperature, dissolved oxygen content, and pH were monitored and recorded continuously using a web-accessible system (Apex AquaController and PM3, Neptune Systems, Morgan Hill, CA, USA). Total ammonia nitrogen was assayed using an ion-selective electrode (Orion DUAL STAR, Thermo Scientific, Waltham, MA, USA) and salinity with a portable refractometer (Pentair Aquatic Eco-systems, Apopka, FL, USA) once weekly. The concentration of un-ionized ammonia nitrogen was calculated per Bower & Bidwell (1978). Water quality

parameter averages were as follows: temperature, 21.7 °C; dissolved oxygen content, 106% saturation; pH, 7.77; total ammonia nitrogen, 0.19 mg/L; unionised ammonia, 0.0029 mg/L; salinity, 31 ppt. Water quality parameter ranges were as follows: temperature, 18.0-24.9 °C; dissolved oxygen content, 87-171% saturation; total ammonia nitrogen, 0.02-0.50 mg/L; unionised ammonia, 0.00037-0.015 mg/L; salinity, 28-34 ppt. Fish were fed once daily, between 1000 h and 1600 h, at a rate of 20-40 kilocalories/kg/day; feed items consisted of previously-frozen Boston squid, (*Loligo pealei*), Northern shortfin squid (*Illex illecebrosus*), Atlantic herring (*Clupea harengus*), Atlantic mackerel, (*Scomber scombrus*), and Atlantic butterfish (*Peprilus triacanthus*). Supplemental vitamins (Sea Tabs®, Pacific Research Laboratories, San Diego, CA, USA) were administered as a weekly ration of approximately 277 mg/kg of bodyweight.

2.1.3. Behavioural Observation of Fish

Qualitative observations of the fish maintained at the BARL facility were recorded daily. Specifically, physical appearance, feeding activity (amount consumed, voracity, preference for particular feed items or sizes), swimming activity (rate, water column position, body posture), and social interaction (schooling, aggression) were observed and recorded for evaluation of potential relationships with the development of puffy snout.

2.1.4. Physical Examination and Necropsies

Clinical signs of puffy snout were evaluated by examination of whole fish – live and dead. Necropsies of all captive fish were completed immediately after the fish were removed from the tank, and within six hours of death. Necropsies of fish within the wild group were completed immediately upon capture and euthanasia. The necropsy procedure for all yellowfin (captive and wild fish) and one Atlantic bluefin was as follows:

- i. Straight fork length (FL) was measured in centimeters (cm) using a standard metric measuring tape and recorded.
- ii. Weight was measured in kilograms (kg) using a Chatillon DHB 50K digital scale and recorded.
- iii. Tissue samples, from epidermis to as deep as possible in cross section (target of 2 cm into skeletal muscle), were obtained from multiple areas of the head and trunk, of both visibly-afflicted and apparently-normal regions. Several sample locations were consistent amongst all necropsied fish based on the presence of lesions: immediately rostral to the eye, the lower jaw, and dorsal to the edge of the operculum. All sample locations were recorded.
- iv. Samples were obtained from liver, kidney, spleen, and gill tissue.
- v. Photographs were taken throughout the course of the necropsy to document external and internal features.
- vi. Quantitative (i.e., number of tissue nodes, extent of eye occlusion in percentage) and qualitative (i.e., colour and texture) extent and appearance of external and internal features were documented.

One whole head from a freshly-euthanized Atlantic bluefin tuna and the fixed tissues from the Atlantic bluefin samples as described above were shipped to Kennebec River Biosciences in Richmond, Maine, USA for histology processing and investigative parasitology and virology. Samples were taken from various locations throughout the whole head and fixed by Kennebec River Biosciences technicians.

All tissue samples were placed in 10% neutral buffered formalin and allowed to fix for at least 72 hours (Mumford, 2004). Formalin-fixed tissue samples were transferred to 70% ethanol for longer-term storage (Mumford, 2004.) Fixed yellowfin tuna and false albacore tissues were shipped to Mass Histology Service in Worcester, Massachusetts, USA.

2.1.5. Histology

Tissues were paraffin-embedded and sectioned at 5 μm . One section from every tissue was stained with hematoxylin and eosin, and selected tissues had an additional section, either directly preceding or directly following, stained with Masson's trichrome.

To examine the effects of puffy snout at the tissue and cellular level, tissue sections were examined using light microscopy (AxioImager M2, Zeiss, Oberkochen, Germany), and photomicrographs taken using the Zeiss AxioImager M2 system digital camera (AxioCam HRc, Zeiss, Oberkochen, Germany). Various features of the tissues were investigated (i.e., cell type, cell number, cell size, cell morphology) such that a 'cellular landscape' of puffy snout syndrome could be produced. To assess specific

tissue abnormalities related to puffy snout, tissue comparisons were carried out between fish with and without puffy snout.

2.1.6. Parasitology and Bacteriology

The Atlantic bluefin tuna head and additional soft tissues sent to Kennebec River Biosciences were visually screened for macroscopic parasites.

Aseptically-sampled tissues from the head, the additional soft tissues, and whole blood were shipped on ice overnight to Kennebec River Biosciences and processed for bacteria cultures. Samples of kidney, spleen, liver, and puffy snout lesion tissue were inoculated onto marine solid media for detecting the presence of *Aeromonads*, *Chryseobacterium*, *Flavobacterium*, *Edwardsiella*, *Listonella*, *Moritella*, *Photobacterium*, *Vibrio*, *Carnobacterium*, *Streptococcus*, *Nocardia*, and *Mycobacterium*.

2.1.7. Virology

Aseptically-sampled tissues sent to Kennebec River Biosciences were processed for virology. Samples of kidney, spleen, liver, and puffy snout lesions were homogenised, diluted in Hank's balanced salt solution, sterile filtered, and inoculated on five different cell culture monolayers. Tuna-specific cell lines were not available, so the five cell lines chosen were those routinely used to screen for viral agents in finfish: Chinook Salmon Embryo (CHSE-214), Epithelioma Papulosum Cyprini (EPC), Bluegill Fry (BF-2), Fat Head Minnow (FHM) and Striped Snakehead (SSN-1). Blind transfers from original plates were completed after seven days incubation.

Original plates were monitored for 28 days. Blind transfer plates were monitored for 21 days.

2.2 *Investigation of Etiology*

To assess the conditions under which puffy snout occurs (and, just as important, does not occur), a survey, with an accompanying introductory letter and photographs depicting puffy snout (Appendix C), were sent electronically to researchers and representatives of institutions throughout the world that have maintained tunas in captivity. In recognition that some researchers and/or institutions/facilities might be hesitant to provide data, the inquiries were designed to maximise integrity of information. The survey inquired about quantitative and qualitative facets of each operation that might be potential causative factors of puffy snout. These include: fish species and size, capture and transport procedures, holding tank physical and environmental characteristics, feeding regime, water quality, incidence of other confounding health factors (pathogenic or physical injury), and others. Individual factors as well as potential interactions were examined by manual comparison of the variable(s) (i.e., holding tank biomass density) and the corresponding facility's prevalence of puffy snout. These relationships were subsequently compared between facilities.

Although the identities of individuals and institutions were requested, surveys were evaluated independently of the identifying information. To ensure blind analysis, identifying information was removed from the survey before analysis.

The institutions of interest were research, commercial, and public-display facilities. Each has maintained, or does currently maintain, scombrid species in captivity.

3. Results

3.1. Fish Condition at Capture, During Transport, and During Holding

As captured fish were intended for long-term holding in the BARL land-based facility, only those in good physical condition as defined in Section 2.1.1 were transferred to the fishing vessel's transport unit. During transport, fish not displaying normal behavioural qualities, were removed from the transport unit and excluded from the study. Fish meeting the physical and behavioural criteria were transferred to the tank at the BARL facility. All fish in this study initiated feeding in captivity between one day and three weeks post-capture, and continued to meet the physical and behavioural criteria until the development of puffy snout.

3.2. Description of Pathology

3.2.1. Clinical Signs

During the course of this study, the first observable symptoms of puffy snout emerged three to seven weeks in yellowfin and six to nine weeks in Atlantic bluefin following collection and transfer to the land-based tank. In both species, symptoms were first seen on the dorsal surface of the head. The skin in this area, which appears shiny and deep blue in colour in wild fish (Fig. 1a), developed a faded, dusty appearance, and began to swell and wrinkle. Three to six shallow “wrinkles” several

centimeters apart developed simultaneously and were longitudinal, beginning just anterior to the eyes and extending posteriorly to behind the eyes. At this stage, there was no accompanying change in either feeding or swimming behaviour, nor in social interaction with con- or heterospecifics.

As the condition progressed, the area just rostral to the each of the eyes, and approximately the diameter of the eye itself, exhibited the most significant signs of swelling or “puffiness” (Fig. 1b). Each of these two areas began to swell such that the incorporated tissue could be distinguished from the surrounding, non-incorporated tissue. Additionally, the area directly ventral to each of the eyes became noticeably textured, with a dense network of wrinkles. A less dense but still noticeable network of wrinkles began to form on the lower jaw (Fig. 1b,c). Because the tissue nodes anterior to the eyes had grown distally to approximately a few centimeters thick, it is presumed that forward vision was impaired at this stage. Furthermore, feeding behaviour often, but not always, became affected. While interest in feed remained largely unchanged, attempts to consume a feed item became less successful; the approach was less direct or aggressive and was often aborted immediately before reaching the feed item, such that the fish either turned away from or swam directly past it. At this stage, no effect on swimming behaviour or social interaction was noticeable.

Further progression was characterised by an increase in both the number of tissue nodes and the surface area affected; the rostral-most part of the snout, the upper and lower jaws, the area posterior to the eyes, the dorsal surface of the head, and the operculum become noticeably ‘puffy’ (Fig. 1c,d). The nodes anterior to the eyes

extended posteriorly, such that the eyes themselves became occluded and less visible from a profile perspective (Figs. 1d,e,f and 2). The area immediately ventral to the eyes typically remained highly textured (Fig. 1d). The vivid colouration on the head and operculum seen in wild fish (Fig. 1a) was lost. In fish with puffy snout, this area darkened in some areas and lightened in others, and there was a loss of the sharp demarcation between colours (dark blue, yellow, and silver in yellowfin and dark blue, light blue, and silver in Atlantic bluefin), which became faded and blurred (Figs. 1c,d,e). At this stage, obvious changes in swimming and feeding behaviours were apparent. Fish began to employ a more irregular tail-beat means of swimming, whereby long coasts followed pulses of one to four successive beats of the tail. Fish often began to pitch nose-down, by approximately 10 to 20 degrees, and list to one side or the other (but always with the dorsal side towards the tank perimeter). Feeding success also continued to decline, and it was not uncommon for a fish to consume no feed on a given day. Some fish maintained interest in feed, while other fish lost interest in feed. Those that maintained interest were often unsuccessful in actually consuming feed items, seemingly due to impaired forward vision. As fish approached a feed item, they often began to ‘stall’ within a meter of reaching the item, while simultaneously opening the mouth. A cessation of forward movement subsequently caused the fish to hastily resume swimming effort, usually without successfully capturing the feed. It was also common for fish to swim directly into a feed item without actually attempting to consume it. As a result of decreased caloric intake, fish began to noticeably lose weight. Changes in swimming and feeding behaviours, however, were observed intermittently at this stage, punctuated with periods of

seemingly normal behaviour. Shifts between normal and abnormal swimming typically occurred on an hourly to daily basis, whereas periods of normal and abnormal feeding each typically lasted days to as many as four weeks. As the condition progressed, abnormalities of both swimming and feeding became longer in duration and/or more frequent. At this stage, fish afflicted with puffy snout began to spend less time swimming in proximity to other fish, but aggression or other displays of social conflict were never observed.

In the late stages of puffy snout (Figs. 1f and 3), the swollen nodes on the head, jaws, and operculum increased markedly in number and extended posterior to the operculum. The eyes became severely occluded, likely impairing vision as evidenced by fish occasionally contacting/bumping the walls of the tank. The operculum also was noticeably thickened. The nose-down pitch of swimming posture became more severe, perhaps as much as 45 degrees, and listing became more frequent and more dramatic. Swimming speed slowed considerably with tail beats less successive and deliberate, and periods of coasting between sequences of tail-beats became longer. Feeding behaviour, both in terms of capture success and interest, continued to decline until it eventually ceased altogether and fish exhibited signs of emaciation.

During the course of the study, clinical signs of puffy snout showed no regression. Mortality of captive group yellowfin occurred after 37, 62, 108, 269, and 299 days in captivity (Table 1). Euthanasia of captive Atlantic bluefin took place after 162 and 161 days in captivity (Table 1).

Of note, the false albacore was held in captivity for approximately 580 days (1 year, 7 months) (Table 1) without developing puffy snout. The other eleven false

albacore held in the BARL facility, which were not used for determination of clinical or cellular-level changes caused by puffy snout, are nevertheless considered important; no clinical or behavioural changes, with the exception of a developed awareness of people standing at the tank, were observed for the duration of their captivity in *any* of the twelve false albacore held,

3.2.2. Histology

3.2.2.1. *Region Anterior to Eye*

Numerous differences in the structure and composition of tissue anterior to the eye were found between wild fish, which did not show signs of puffy snout, and captive fish with puffy snout. Additionally, individual variation was apparent in the severity of the condition between captive fish.

The overall density of tissue in this area was reduced with the development and progression of puffy snout. Tissue in wild fish was tightly-compacted and very little area remained unstained (i.e., colourless) when stained with H&E (Figs. 4a, 5a, and 7b). In fish that developed puffy snout, a greater proportion of the tissue section remained unstained, demonstrating a less dense network of tissue. The unstained proportion was positively correlated with the severity of the condition. For example, tissue from an Atlantic bluefin tuna (Fig. 3), which had the most severe clinical signs in the study, showed more unstained area (Fig. 4c) than did a yellowfin tuna (Figs. 1f, and 4b) with a less severe affliction. Progression of reduced tissue density is apparent

when comparing a wild fish euthanized post-capture (Fig. 5a) to one that died after 62 days in captivity (Fig. 5b) to another fish that died after 299 days in captivity (Fig. 5c).

Not only did the density of tissue change when fish developed puffy snout, but so too did the composition. The tissue anterior to the eye in wild group fish was dominated by uniform, dense muscle, as evidenced by its red colouration after staining with Masson's trichrome (Figs. 8a,c and 9). In the uppermost regions of the tissue, muscle fibers were almost exclusively dominant. More proximally, networks of collagen fibrils, which provide structural integrity, were interspersed with muscle tissue. Collagenous tissue comprised approximately 30-40% of an entire top-to-bottom cross section of tissue in this region (Figs. 8a,c). In contrast, fish that had developed puffy snout were characterised by the apparent necrosis of muscle tissue and replacement with collagen (Figs. 8b,d and 9), as evidenced by blue staining with Masson's trichrome. Few, isolated muscle fibers remained (Fig. 9b). Additionally, as mentioned, the resulting network of all tissue is less dense than that which it replaced.

In wild fish, what appeared to be mucous-producing goblet cells in the epidermis were well defined and covered only by a thin layer of smooth muscle and perhaps a 1-2-cell-thick layer of epithelial cells. In fish that developed puffy snout, there was an apparent degradation of these cells. The dark colouration normally found around the perimeter of the cells (Figs. 4a, 5a) disappeared (Figs. 4b, 5bc). In severe, late-stage afflictions, goblet cells were infiltrated by connective tissue (Figs. 4b, 5c) and appeared to dissolve entirely in the most severe cases (Fig. 4c). The layer of tissue above these cells remained, though it appeared to become collagenous (Fig. 9b), and

the overlying layer of epithelial cells may, in some instances, become markedly thickened (Fig. 6) and may also have contained numerous lipid clusters (Fig. 6b).

Deeper in the cross section, the tissue of wild fish remained an organised matrix of smooth muscle with abundant nuclei (Fig. 7b). As in upper regions for fish with puffy snout, much of this muscle was replaced with collagenous tissue (Fig. 7a,c). The matrix of connective and muscle tissue was loose, seemingly random, and highly disorganised. Nuclei were less numerous than in wild fish. Also similar to upper regions, more unstained area was visible in fish that developed puffy snout when compared to wild group fish.

The tissue taken from the anterior to the eye in the captive false albacore (Fig. 12a) showed both similarities and differences to tissue taken from captive fish which had developed puffy snout. Trichrome staining revealed the dominant presence of collagenous tissue, as was seen in fish with puffy snout. However, the collagen was tightly-packed with very little interstitial space when compared to fish with puffy snout, and muscle fibers were interspersed throughout, becoming more numerous with proximal progression. Beneath this region was a layer of well-defined adipocyte clusters, infiltrated by seams of interwoven collagen and muscle tissue.

3.2.2.2. *Dorsal Musculature*

Differences in composition and structure were found between dorsal musculature tissue of wild yellowfin tuna (Fig. 10a) and captive yellowfin tuna that developed puffy snout (Fig. 10b). The most marked difference was in the epidermal region. Wild fish had a muscular epidermis with very little collagen interspersed. In fish that

developed puffy snout, a prominent, thick collagen layer was most axial. This collagen was more densely packed nearer the surface, but in no areas as loose as in some tissues anterior to the eyes of puffy snout-afflicted fish (Figs. 6b and 8d).

Interestingly, a different phenomenon was observed in dorsal musculature tissue of two yellowfin (Fig. 11) that displayed signs of puffy snout. Tissue was excised from a less visibly-afflicted, more posterior location – between the first and second dorsal fins – (Fig. 11a) and from a more afflicted, anterior location – the leading edge of the first dorsal fin (Fig. 11b). However, rather than collagenous growth in the epidermal region (Fig. 10b), it appears that a proliferation of collagen may occur in the hypodermal region. In the lesser-afflicted tissues (Fig. 11a), it can be seen that a relatively thin layer of collagenous tissue exists between the skeletal muscle and the dermis. In the more afflicted tissue (Fig. 11b), this hypodermal region is markedly thicker, perhaps by as much as three-fold, as a result of increased collagen deposition. The collagen, as seen in much of the tissue anterior to the eye, is not densely packed, but rather has more interstitial space. The dermis in the more afflicted tissue (Fig. 11b) is also thicker than that of the lesser-afflicted tissue (Fig. 11a), seemingly due to several layers of collagen laid down on top of one another. Melanocytes are present in both tissue samples and do not appear to differ between them.

As seen in tissue anterior to the eye, dorsal musculature tissue from the captive false albacore (Fig. 12b) showed similarities and differences to captive group fish that developed puffy snout. Beneath a thin collagenous ‘skin’ and seemingly-intact goblet cells, the epidermis of the false albacore was comprised of layered, ovular collagen bundles. The collagenous epidermis in false albacore was dissimilar in composition to

the muscular epidermis in wild yellowfin (Fig. 10a), but in structure and relative thickness seen in captive yellowfin with puffy snout (Fig. 10b). In the false albacore, a thin collagenous basement membrane separated the hypodermis, a thick adipose layer with scattered muscle fibers and collagen fibrils, from the underlying skeletal muscle (Fig. 12b).

3.2.3. Parasitology

Parasite screening of the head of the Atlantic bluefin with puffy snout revealed the presence of parasites on gill filaments and evidence of parasitic activity at the base of gill arches. The organisms observed on filaments were identified as trematodes, likely Didymozoids. At the base of gill arches, nodules were observed that are likely associated with the presence of *Cardicola* species. Evidence of parasites was present at 1-2 organisms/nodules per gill arch.

3.2.4. Bacteriology

Screenings of captive Atlantic bluefin tissues were negative for *Aeromonas*, *Chryseobacterium*, *Flavobacterium*, *Edwardsiella*, *Listonella*, *Moritella*, *Photobacterium*, *Vibrio*, *Carnobacterium*, *Streptococcus*, *Nocardia*, and *Mycobacterium* species in kidney, spleen, liver, and head tissues, as well as blood. *Pseudomonas* was detected in one blood sample, which was isolated from sample tubes containing excised soft tissue; sequence analysis of the 16S rRNA gene showed closest sequence homology, at 99%, in GenBank to *P. fluorescens* and *P. jessenii*.

3.2.5. Virology

Cytopathic effect (CPE) was not observed with kidney, spleen, liver, or puffy snout lesion tissue homogenates in CHSE-214, EPC, BF-2, or FHM cell lines. After 22 days incubation, CPE-like abnormalities were observed on SSN-1 cells inoculated with each of the four tissues. Serial dilutions were prepared and monitored for four additional weeks, but no further changes indicative of a transferrable viral agent were observed.

3.3. Investigation of Etiology - Survey

3.3.1. Responding Facilities

Eleven of 28 facilities returned a completed survey. One facility had ceased tuna research activities but reported on the time frame when fish were held. The remaining ten facilities were actively engaged in research on tunas. Two of these facilities are also engaged in public display, and another two have direct commercial application and operation.

Seven facilities reported to have reared only one scombrid specie in captivity: three held yellowfin (*Thunnus albacares*), three held Atlantic bluefin (*T. thynnus*), and one held blackfin (*T. atlanticus*). The other four facilities reported to have each held more than one scombrid species in captivity. Three of these facilities each held three scombrids in captivity concurrently: one held yellowfin, Atlantic bluefin, and false

albacore (*Euthynnus alleteratus*), another held Atlantic bluefin, false albacore, and Atlantic mackerel (*Scomber scombrus*), and a third held yellowfin, kawakawa (*E. affinus*), and skipjack (*Katsuwonus pelamis*). A fourth facility held five scombrids in captivity: yellowfin, Pacific bluefin (*T. orientalis*), kawakawa, Pacific mackerel (*S. japonicus*), and Pacific bonito (*Sarda chiliensis*). Of the 11 total facilities, seven (64%) reported puffy snout to have occurred at least once.

In addition to some facilities rearing more than one species, one facility reported different degrees of prevalence of puffy snout between two different rearing units; as such, each of these rearing units at this facility was evaluated independently. Taking into account these tanks, 22 occurrences of a scombrid species being held in captivity were reported. Puffy snout occurred in 15 (68%) of these.

3.3.2. Biological Data

3.3.2.1. Species

Of the ten scombrid species held in captivity (four *Thunnus* species (yellowfin, blackfin, Pacific bluefin, Atlantic bluefin), two *Euthynnus* species (kawakawa, false albacore), one *Katsuwonus* species (skipjack), two *Scomber* species (Pacific mackerel, Atlantic mackerel), and one *Sarda* species (Pacific bonito)) nine (90%) were reported to have developed puffy snout syndrome at least once; only false albacore was reported to have never developed the condition (Table 2).

In yellowfin, puffy snout occurred in six of seven (85%) reports in 1%, 10%, 20%, 20%, 80%, and 100% of fish held. In Atlantic bluefin, puffy snout occurred in

two of five (40%) reports, each with 100% prevalence. The single report of Pacific bluefin stated 20% prevalence and prevalence in the single report of blackfin was 80-90%. One of two (50%) reports of kawakawa stated puffy snout was observed, with 10% prevalence. Puffy snout was not observed in false albacore at either of two facilities. The single reports of skipjack and Pacific mackerel indicated 100% and 20% prevalence, respectively. Similarly, prevalence in Atlantic mackerel was 100%. The single report of Pacific bonito indicated a prevalence of 20%.

3.3.2.2. Size

Scombrids across a wide size range were reported to have been held in captivity; based on the sizes reported, it is assumed that larvae, fingerlings, juveniles, post-juveniles, and fully mature adults were reared at the participating facilities. Across all species, fish from 15 to 150 cm forked length (FL) and <1 to 60 kg, were reported to have suffered from puffy snout. Fish held without developing puffy snout were between 10 and 200 cm and <1 to 400 kg (Table 2).

An overlap in size between fish that developed and did not develop puffy snout was reported for four species (Table 2). In yellowfin, puffy snout occurred in fish 40-150 cm FL and 1-60 kg, and did not occur in fish 20-200 cm FL and 0.5-168 kg. At the only facility that held Pacific bluefin, it was reported that fish 50-150 cm FL and 6-60 kg did and did not suffer from puffy snout. Both Pacific mackerel and Pacific bonito developed and did not develop puffy snout at 15-35 cm FL.

Skipjack and Atlantic mackerel, each reported with 100% puffy snout prevalence, were reported to be 40-50 cm FL and 1-3 kg, and 45 cm FL of unspecified

weight, respectively. As the only species to have been reported never to develop puffy snout, false albacore ranged in size from 40-60 cm FL and 2-5 kg.

Two of 10 (20%) reports of puffy snout occurring at least once but in less than 100% of fish showed no overlap in size between afflicted and non-afflicted fish. In blackfin, it was reported that fish less than 50 cm FL and 3 kg did not develop puffy snout, whereas those larger, did. For one report of kawakawa, two size classes were held; fish which were 50-65 cm FL and 1-5 kg did suffer from puffy snout, but fish which were 10-15 cm FL and less than 1 kg did not. A second report of kawakawa of 40-50 cm FL and 1-3 kg indicated puffy snout did not occur in these fish. Two positive reports of puffy snout in Atlantic bluefin occurred in fish 70-130 cm FL and 8-45 kg, and three negative reports were given by facilities holding fish less than 15 cm FL, 3-100 g, or 50-400 kg, respectively.

3.3.2.3. Sex Ratio

Only one facility recorded the sex of puffy snout-afflicted tunas; for blackfin, a 50:50 male:female ratio was reported.

3.3.2.4. Cohabitation of Heterospecifics & Afflicted/Non-Afflicted Fish

Three of the eleven (27%) facilities reported holding more than one species simultaneously in a common tank. Of these, one held yellowfin and Pacific bluefin together, one held yellowfin, skipjack and kawakawa together, and one held false albacore with both Atlantic bluefin and yellowfin, but never all three at the same time.

Eleven of 15 (73%) positive reports of puffy snout indicated there were fish that never developed the condition when held with conspecifics that already had or eventually developed it. Five of these occurred in yellowfin, and one each in Pacific bluefin, blackfin, kawakawa, skipjack, Pacific mackerel, and Pacific bonito. The four reports of all conspecifics developing the condition occurred twice in Atlantic bluefin and once each in yellowfin and Atlantic mackerel. Of the nine reports of heterospecifics being held together, seven (78%) indicated that individuals of one species never developed puffy snout when individuals of another species did. Only two reports indicated that all members of one species (kawakawa and false albacore) showed no clinical signs of puffy snout while individuals of another species in the same tank developed it.

3.3.2.5. Time in Captivity Until Appearance of Clinical Signs

All but two incidences of puffy snout were reported to have occurred six months or less after fish were reared in captivity (Table 3). Once, for Atlantic bluefin, it developed 9-12 months post-introduction to the holding tank, and once, for yellowfin, it occurred only after two years in captivity. The most common finding indicated that puffy snout arose within 1-4 months. In blackfin, it reliably developed after one month in captivity. The shortest time to development occurred in skipjack at 2-3 weeks.

3.3.2.6. *Presence in Wild Fish*

The representatives from all 11 facilities reported to have never seen puffy snout in wild fish – either those handled and transported, angled and released, or observed at sea.

3.3.2.7. *Presence in Captive Progeny*

Five of the 11 facilities reported to have reared captively-spawned tuna progeny. Four (three rearing yellowfin and one rearing Atlantic bluefin) reported never observing puffy snout in these progeny, and one, rearing Atlantic bluefin, could not report with complete confidence the absence of puffy snout in these progeny.

3.3.3. *Capture and Transport Data*

3.3.3.1. *Capture Method*

All but two reports used traditional angling (hook and line) to capture wild fish (Table 4). Rod and reel was reported most often and for every species, with handlining reported twice for yellowfin and a liftpole reported once each for yellowfin, Pacific bluefin, kawakawa, and skipjack. The two reports not using traditional angling methods, both for Atlantic bluefin, employed purse seine capture.

3.3.3.2. *Times Handled*

The two facilities that never handled fish during the capture and transport process utilised purse seine capture. All other facilities handled fish between one and four

times, with 1-2 being the most common. One report of yellowfin handled fish either three or four times, another handled fish two or three times, and a third handled fish three times. Pacific bluefin and one report of kawakawa each handled fish three times (Table 4).

3.3.3.3. Transport Unit Volume & Shape

The volumes of transport units ranged from 0.2-58,873 m³ (Table 5). Two groups employed purse seine capture and conducted at-sea transfers of fish from the seine to floating sea cages, which were towed from the fishing grounds to the holding site. The volumes of the cages were 49,087 and 58,874 m³. All other transport units were 18.5 m³ or less. The two smallest units were used for yellowfin, and were 0.2 and 0.6 m³. Four reports each used two different units, in series, during the course of transport; two used 1.1 and 3.35 m³ units (for both Atlantic bluefin and false albacore), and two used 8 and 11.4 m³ (for yellowfin, Pacific bluefin, and kawakawa). One report for yellowfin used either 1.4 or 1.7 m³ units. Three reports used 2 m³ units (once each for yellowfin, Atlantic bluefin, and false albacore) and four used 6 m³ units (twice for yellowfin and once each for kawakawa and skipjack). Blackfin were transported in either 1.13 m³ units or in a 'Tuna Tube' of unspecified volume. Excluding the sea cage transports, the largest units were used in one report of yellowfin, and were 18 and 18.5 m³.

Round transport units were reported eight times. Oval units were reported seven times. Rectangular units were reported six times. Three reports included the use of more than one shape.

3.3.3.4. Biomass Density During Transport

The biomass density inside transport units was variable both between and within facilities, and ranged from 0.5 to almost 50 kg/m³ (Table 6). In eleven of sixteen cases, however, tunas were maintained at densities of 6.2 kg/m³ or less. The report which had the lowest typical density was for yellowfin, at 0.5-1.5 kg/m³, though at times the densities increased to 5 kg/m³. Five (three for yellowfin and one each for kawakawa and skipjack) indicated densities of 1.6-6.2 kg/m³, with the most typical density being 2.5 kg/m³. One facility reported transporting yellowfin at densities of 0.6-4 kg/m³. Four other facilities employed densities between 2 and 5 kg/m³; 2 kg/m³, 2-4 kg/m³ and 5 kg/m³ for three Atlantic bluefin reports, respectively, and <5 kg/m³ for blackfin. Four reports had the low end of their transport density range at 5 or 6 kg/m³, but two had an upper limit of 10 kg/m³ and two an upper limit of 25 kg/m³. The highest reported density was for yellowfin, and was 25-<50 kg/m³.

3.3.3.5. Number of Fish Transported

The per-trip number of fish transported ranged from 1-600 (Table 6). One of the two reports of sea cage transport stated that 400-600 Atlantic bluefin were transported per cage. Four reports (two for yellowfin and one each for kawakawa and skipjack) stated that 10-20 fish were transported per trip. One report of false albacore stated that 7-10 fish were transported per trip, and one report of yellowfin and the only report for Pacific bluefin indicated that 6-8 fish were transported per trip. One yellowfin report transported 3-6 individuals at a time. Two reports for yellowfin, two for Atlantic

bluefin, and one for blackfin all transported between one and four fish per trip. A single report for yellowfin indicated that only one fish per trip was ever transported.

3.3.3.6. *Size of Fish Transported*

The largest fish transported, Atlantic bluefin, averaged 250 kg each. Two reports of yellowfin stated that transported fish were 7-19 and 8-25 kg, respectively, and the single report of Pacific bluefin reported the fish to be 7-19 kg. Two Atlantic bluefin reports stated that fish were 7-10 and 10-12 kg each. One report of yellowfin stated that fish were 1.8-10 kg each. Most of the reports (four for yellowfin and one each for kawakawa, false albacore, and skipjack) transported fish between 0.5 and 3.6 kg each, though one of those, for yellowfin, reported that fish of up to 10 kg have been transported. The widest size range of transported fish was given by the report of blackfin and stated to be 0.19-14 kg (Table 6).

3.3.3.7. *Time of Transport*

Eight reports (five for yellowfin and one each for blackfin, kawakawa, and skipjack) indicated that fish were transferred to a holding/rearing unit less than three hours after their capture. Two reports (one for Atlantic bluefin and one for false albacore) indicated the elapsed time to be 3-8 hours. One yellowfin report stated transport time to be 14-30 hours. One report each for yellowfin, Pacific bluefin, and kawakawa indicated that transport time was 1-5 days, and two reports for Atlantic bluefin indicated that transport lasted 5-10 days. Two other reports, one for Atlantic bluefin and one for false albacore, stated that transport lasted approximately two days,

but fish were often placed in an intermediate holding tank for up to one month before being moved into their final holding tank (Table 5).

3.3.4. Holding Systems

3.3.4.1. System Type, Holding Unit Volume & Shape

All but three facilities were land-based holding systems. Two of those exceptions were for Atlantic bluefin, held in net pens in open, nearshore locations, and one was for false albacore, held in a caged pen in a harbour.

The volume of long-term holding units ranged from 46 m³ to 58,875 m³ (Table 7). The largest two (49,087 and 58,875 m³) were stocked with Atlantic bluefin. Two other units used for yellowfin exceeded 1,000 m³: one was 1,360 m³ and another was 1,526 m³. One yellowfin report used a 250 m³ tank, and one Atlantic bluefin used a 350 m³ tank. One report each of yellowfin, Pacific bluefin, and kawakawa used both a 110 m³ and a 330 m³ tank. Blackfin were held in an 80 m³ tank, and one report of false albacore used an 83 m³ cage. Yellowfin, Atlantic bluefin, and false albacore were each reported once to be held in a 75 m³ tank. Two reports of yellowfin stated they were held in 46 m³ and 66 m³ tanks, and one report each of kawakawa and skipjack stated they were held in both 46 m³ and 66 m³ tanks.

All but five holding units were round. One facility rearing yellowfin, Atlantic bluefin, and false albacore used round-tanks that had a central column, such that a doughnut shape resulted. The five non-round holding units were all rectangular (Table 7).

3.3.4.2. System Water Re-use and Daily Turnovers

Seven reports used single-pass water flow with no reuse. One report submitted that some water recirculation was used but did not specify to what degree. One report each for yellowfin, Pacific bluefin, and kawakawa stated a reuse of 100%. One yellowfin report stated a reuse of 95%. The single report of blackfin stated a reuse of 70-90%. Yellowfin, Atlantic bluefin, and false albacore were each held in a system with 60-70% reuse. Of all the reports using some reuse, the lowest degree was 50% in one report of yellowfin (Table 8).

The three facilities with open-water holding systems did not indicate an estimated volume of daily water exchange, nor did three reports of land-based holding systems. Measured as the number of full holding unit turnovers per day, the highest rates were 12 for yellowfin, Pacific bluefin, and kawakawa at one facility, and 10-11 for yellowfin, Atlantic bluefin, and false albacore at another facility. Two reports of yellowfin and one each of kawakawa and skipjack had 10. One report of yellowfin had 7-8. The two lowest were 2-3 and 3.5, for yellowfin and blackfin, respectively (Table 8).

3.3.4.3. Influent Water Filtration & Treatment

Of the reports using some degree of water reuse, all but two indicated the consistent use of biofiltration to remove ammonia, the primary nitrogenous metabolite, from the system. One report, for Atlantic Bluefin, stated that biofiltration was never implemented. The second, for false albacore, stated that biofiltration was eventually implemented, but fish were held for more than one year without it (Table 8).

Only five reports specified the minimum particle size removed from influent water (Table 8). One report each of yellowfin and blackfin removed particles down to 20 μm . One report of Atlantic bluefin used 50 μm filtration, one report of yellowfin used 5 μm filtration, and one report of false albacore used both 50 μm and 5 μm over two long-term periods, respectively.

Nine reports indicated that influent water was treated with ultraviolet light (UV) (Table 8). Four of these were for yellowfin and one each was for Atlantic bluefin, Pacific bluefin, blackfin, kawakawa, and false albacore. The other six land-based reports, which did not use UV treatment were for yellowfin (three reports), Atlantic bluefin, kawakawa, and skipjack (one report each).

3.3.4.4. Holding Unit Water Quality

Atlantic bluefin were held in temperatures of 13.5-28, 15-27, 18-24, and 22-25° C. Pacific bluefin were held at 20° C. The facilities holding yellowfin reported water temperatures of 19-24, 20, 20-30, 23-25, 23-25, 24-26, and 27-29° C, respectively. Blackfin were held at 20-30° C. The two reports of kawakawa indicated they were held at 20 and 23-25° C, respectively. False albacore were held in 18-24 and 22-24° C respectively, and skipjack were held at 23-25° C (Table 9).

The dissolved oxygen (DO) content ranged from 65-250% saturation (Table 9). Ten reports (three for yellowfin, two each for Atlantic bluefin and kawakawa, and one each for Pacific bluefin, false albacore, and skipjack) maintained DO at 98-100%. The single report of blackfin maintained 100-250% saturation, the widest range reported. One report each for yellowfin, Atlantic bluefin, and false albacore

maintained 80-150% saturation, though 90-100% was reported to be more typical. Three reports, all for yellowfin, were 85-100%, 90%, and 65-107% of saturation.

The pH in holding units ranged from 7.3-8.3 (Table 9). The widest range at a facility was 7.3-8.2 for one each of yellowfin, Atlantic bluefin, and false albacore. All other reports ranged from 7.6-8.2, with the only exception being one facility that reported a maximum pH of 8.3.

The concentration of un-ionised ammonia (NH₃) in holding units ranged from 0.0024 mg/L to <0.03 mg/L (Table 9). One report for yellowfin reported NH₃ to be “too low to measure,” and two additional facilities, one each for yellowfin and Atlantic bluefin, reported NH₃ < 0.004 mg/L. One report of Atlantic bluefin maintained NH₃ below 0.01 mg/L. One report for blackfin stated NH₃ did not exceed 0.02 mg/L. Three reports, one each for yellowfin, Pacific bluefin, and kawakawa, maintained NH₃ ≤ 0.03 mg/L.

The salinity in holding units ranged from 26-39 ppt (Table 9). The greatest range, 30-36 ppt, was reported for blackfin. Four other reports had a range of 5 ppt; one for yellowfin reported 26-31 ppt and one each for yellowfin, Atlantic bluefin, and false albacore reported a range of 28-33 ppt.

3.3.4.5. Holding Unit Biomass

Four facilities, reporting ten cases of rearing captive tunas, maintained biomass in holding units at 0.5-1.5 kg/m³. Three of these cases (all yellowfin) never exceeded 1 kg/m³, five (yellowfin, Pacific bluefin, kawakawa, skipjack, and Pacific bonito) always exceeded 1 kg/m³, and two (Atlantic bluefin and false albacore) had a range

which extended above and below 1 kg/m³. The density of blackfin also never exceeded 1 kg/m³, and was maintained as low as 0.25 kg/m³. One yellowfin report ranged from 1-1.6 kg/m³, and three others (for yellowfin, kawakawa, and skipjack) ranged from 0.3-1.5 kg/m³. Two Atlantic bluefin reports were, on a consistent basis, the highest; both exceeded 2 kg/m³ and one had an upper limit of 5 kg/m³. One report of yellowfin was the most variable, and ranged from 0.3-7 kg/m³ (Table 7).

3.3.5. Feeding

3.3.5.1. Feeding by Captive Fish

Some 30-100% of fish were collected from the wild began feeding in captivity. Eight reports indicated 100% of fish began feeding; three for Atlantic bluefin, two for yellowfin, and one each for Pacific bluefin, kawakawa, and false albacore. Once, for yellowfin, it was reported that 90% of fish began feeding. Four reports estimated 50-75%; two for yellowfin and one each for kawakawa and skipjack. Another four reports for yellowfin, Atlantic bluefin, blackfin, and false albacore, indicated 50%. The lowest percentage of fish to have begun feeding in captivity was, for yellowfin, 30-40%.

None of the positive reports of puffy snout observed a difference in time-to-first-feeding between fish that never developed puffy snout and those that did. All but two facilities reported to have fed a combination of squid and some type of baitfish (i.e., anchovy, herring, mackerel, sardine, butterfish); the remaining two reported to have

fed only baitfish (Table 10). Six reports stated to have fed previously frozen feed items, and one stated to have fed fresh feed items (Table 10).

Feeding rates were variable (Table 10). Two facilities fed to satiation daily, and one fed six days per week. Two facilities fed a specific daily caloric intake per kilogram of fish; either 30 or 32 kilocalories/kg/day for yellowfin, Atlantic bluefin, Pacific bluefin, false albacore and kawakawa. Three facilities fed based on percentage of fish bodyweight. Two reports of yellowfin indicated that fish were fed 1-10% and 5-10% of the tank biomass per day. One facility reported blackfin were fed 4-12% of the tank biomass per day.

Three facilities did not incorporate supplemental vitamins into the feeding regime: one for Atlantic bluefin, one for yellowfin, Atlantic bluefin, and false albacore, and one for yellowfin, kawakawa, and skipjack. Six facilities incorporated a vitamin complex supplement: one did not specify an administration rate, two specified a rate of once weekly, and three specified a rate of once daily. All reports of daily administration occurred for yellowfin. One of these incorporated vitamin C and vitamin E in addition to the complex, and one quantified the daily dose to be a complex at 1% of feed weight and vitamin C at 500 milligrams/fish (Table 10).

None of the positive reports of puffy snout indicated to have observed a difference in time-to-first-feeding between fish that never developed puffy snout and those that did.

4. Discussion

4.1. Description of Pathology

4.1.1. Clinical Observations

Puffy snout impacts, directly and indirectly, several functional processes. As the condition progresses, the increasingly-swollen nodes of tissue restrict the visual field, negatively impacting feeding, and later, swimming. When interest in feed is still apparent during early stages of puffy snout, restricted vision and movement of the jaw results in a decline in capture and ingestion success. The periodic declines in interest in feed seen in moderate and severe afflictions may be related to the physiological process of coping with the stress associated with puffy snout and/or the causative factors, and may be further compounded as less feed is ingested. It is unclear what might initiate a resumption of interest in feed, but the continuation of a resumed interest may be similarly linked to regularity of gastric processes. During the course of the study, no mature gonads were found in captive group fish. While it cannot be confirmed that wild fish of similar (species-specific) sizes in the western north Atlantic are sexually mature, it could be assumed that the same stressors – and stress-coping mechanisms – that might play a role in the reduction of feeding interest would similarly have negative consequences for reproductive physiology. Indeed, several others factors (e.g., poor water quality, improper exogenous cues, lack of mature mates) could inhibit gonad maturation and/or spawning, but if puffy snout affects feeding success, it seems likely that reproductive processes would also be inhibited.

Nevertheless, an inconsistent supply of nutrients due to periodic declines in feed consumption would result in poor development of gonads and gametocytes. Finally, the physiological load of coping with puffy snout and its causes may negatively impact immune function. While there was no evidence of increased bacterial, viral, or parasitic load, nor were superficial abrasions healed incompletely or ineffectively, it nonetheless seems possible that some degree of immunodeficiency was present.

It was not, however, apparent that puffy snout impacted social interaction; no aggression or isolation behaviours were observed. But considering the aforementioned possibility of reproductive dysfunction, it is reasonable to suggest that fish afflicted with puffy snout that would otherwise be likely spawners may not properly mature, or, either by choice or by exclusion, participate in spawning.

4.1.2. Histology

Previous investigations (Tester, 1952; Nakamura, 1972) suggested that puffy snout causes swelling in the tissues affected. With the assumption that the term “swelling” was used to define the process of tissue inflammation, this was examined during histological investigation. While certain aspects of tissue histology, particularly those in tissues anterior to the eye, suggest an inflammatory response, other aspects typical of inflammation are absent. For example, the unstained area observed in these tissues (Figs. 6b,c, 8b, 10d and 11b) suggests the presence of either fluid or lipid. Because those areas lack observable cytoplasmic membranes that are typical in clusters of adipocytes, it is likely that fluid, not lipid, occupied the interstitial, unstained space between collagen fibrils. As fluid accumulation, or edema,

is a hallmark of the inflammatory response (Secombes, 1996; Roberts, 2001), this observation supports the notion that puffy snout is the manifestation of inflammation.

In contrast, when excised during the necropsy procedure, the tissues showed no evidence of having a functional blood supply, and when viewed with microscopy, showed no evidence that basic structural integrity of the tissue was maintained – both of which are benchmarks of the inflammatory response (Secombes, 1996; Roberts, 2001). In addition, there is a notable absence of cells indicative of inflammation. However, those functional indications of the presence of an inflammatory response may have been present earlier in the progression of the condition. If the inflammatory response surpassed the acute phase and transitioned into the chronic phase prior to the time of necropsy, cells that are more closely associated with acute inflammation (i.e., neutrophils) would not be expected to be present. But at the time of necropsy, puffy snout – and the inflammatory response – may have progressed sufficiently that cells associated with chronic inflammation (i.e., monocytes, macrophages, lymphocytes) may have given way to a fibrotic condition, as observed in earlier research on the inflammatory response (Roberts, 2001; Wynn, 2008), and therefore no longer present.

The observed proliferation of collagen, accumulation of excess extracellular material, and its status as a successor to chronic inflammation (Roberts, 2001; Wynn, 2008; Wynn and Ramalingham, 2012) provide evidence for the occurrence of fibrosis in at least marginally-progressed puffy snout. The idea that puffy snout becomes a fibrotic condition is further supported by the loss of functional blood supply in underlying tissues. But perhaps most importantly, fibrosis carries the potential for reversal if the irritant is removed (Wynn and Ramalingham, 2012). Indeed, Nakamura

(1972) reported that for kawakawa held in raceway tanks, the puffy snout that had developed receded, or “disappeared”, within two weeks of transfer to a larger tank.

The findings suggest that while the inflammatory response process may occur in the early stages of a puffy snout, the cellular composition and its lack of functional blood supply are not indicative of an active and ongoing inflammatory response. Based on the observed and marked proliferation of collagen in puffy snout-afflicted tissues, the condition appears to be fibrotic in nature, at least in later stages.

Of note, the tissues anterior to the eye in captive group false albacore were more similar in composition to captive group yellowfin that developed puffy snout (i.e., primarily collagenous) than to wild group fish that did not. However, the differences in structure from puffy snout tissues and the complete absence of any clinical signs of puffy snout in the false albacore suggest species-specific differences in ‘normal’ tissue composition. Because of the absence of puffy snout in false albacore, these differences may result in that species being less sensitive to stressors or irritants that induce the development of puffy snout in other tunas. For example, perhaps differences in lateral line morphology, or the number or sensitivity of receptor cells on the head region render false albacore more able to cope with the conditions that other tunas in this study were impacted by. Regrettably, no samples of wild false albacore were taken, which would have afforded a conspecific comparison between wild and captive fish.

4.1.3. Parasitology, Bacteriology, & Virology

While parasite presence was observed on the gills of the Atlantic bluefin specimen examined, the specific pathogens and burden were low, consistent with those previously reported in captive and wild tunas. Didymozoid trematodes have been isolated from tunas in captivity (Munday et al., 2003; Al-Bassel and Ohaida, 2006; Nowak et al., 2006; Mele et al., 2010), including Atlantic bluefin (Mladineo, 2006; Mladineo and Bočina, 2009; Mladineo et al., 2011), so their presence in the specimen examined here is of no surprise. Furthermore, they have been considered to not elicit a strong cellular response (Mladineo, 2006) and therefore would likely not result in the marked changes in the head region that were observed here. *Cardicola* species are common in Pacific and Southern bluefin tunas (Munday et al., 2003; Deveney et al., 2005; Nowak et al., 2006; Kirchoff, 2012) and have also been observed in Atlantic bluefin (Bullard et al., 2004; de Ybañez et al., 2011), yellowfin, and bigeye (Yamaguti, 1970; Smith, 1997). In cage-reared Pacific and Southern bluefin, high (Aiken et al., 2006; Dennis et al., 2011) and low (Colquitt et al., 2001; Deveney et al., 2005; Aiken et al., 2006), *Cardicola* infections have produced a different set of immune responses [(e.g., respiratory distress, lethargy (Munday et al., 2003)] and clinical signs than puffy snout. One study of wild juvenile Atlantic bluefin in the Bay of Biscay found 98% of fish examined to be hosting parasites, including Didymozoids (Rodríguez-Marín et al., 2008). Though all captive tunas referenced above were held in open-water net pens, the presence of both Didymozoid and *Cardicola* pathogens in captive and in wild fish suggest little contribution of these parasites to development of puffy snout in Atlantic bluefin.

Despite the observance of *Pseudomonas* bacteria in one blood sample, it is not anticipated to be the causative agent of puffy snout. *Pseudomonas* species, *P. fluorescens* in particular, are ubiquitous among freshwater and saltwater fish species and among wild and farmed fish (Austin and Austin, 2007; Khalil et al., 2010; Austin and Austin, 2012), and has been isolated from Atlantic bluefin in the Adriatic Sea (Kapetanovic et al., 2011). However, a typical *Pseudomonas* infection is associated with necrotic and/or haemorrhagic lesions (Schäperclaus, 1979; Ahne et al., 1982; Sakai et al., 1989; Austin & Austin, 2007; Austin & Austin, 2012), and the lesions associated with puffy snout are neither entirely-necrotic or haemorrhagic. Ultimately, the presence of *Pseudomonas* bacteria in the blood is likely to be a result of contamination during collection or processing, though the source is unknown. Other bacteria isolated from cultured Atlantic bluefin include those from the genera *Brevundimonas*, *Moraxella*, *Pasteurella*, *Staphylococcus*, *Klebsiella*, *Weeksella* and *Vibrio* (Kapetanovic et al., 2011).

Although no compelling evidence of cytopathic effect (CPE) was observed in the five fish cell lines inoculated with tissue homogenate from puffy snout affected tissue, a viral agent cannot be eliminated as a cause of puffy snout. Because tuna-specific cell lines could not be obtained, the cell lines used were those routinely employed for viral screening in marine and freshwater finfish (Peters, 2004). While SSN-1 is the most promising candidate of those used in this study because of its initial CPE-like activity, more definitive results would require the use of tuna specific cell lines.

4.2. *Investigation of Etiology – Survey*

As with any survey, there may have been inherent bias in the provision of information by the respondents. However, as the survey was developed to minimise such bias, it is not currently believed that the results of this study were significantly compromised. A high degree of confidence in the accuracy remains in the data provided.

4.2.1. Biological Data

Several factors related to the biology of the tunas themselves and other characteristics of the environment in which they were held were found to have unclear contributions to the development of puffy snout (Table 11). The species, size, and sex of a fish, and whether that fish was wild-caught or spawned and raised in captivity, are all factors to be determined.

All species examined except false albacore developed puffy snout. The idea of species-specific susceptibility to puffy snout was first communicated by Nakamura (1972), who reported that skipjack developed the condition more frequently than yellowfin. It is notable that at the University of Rhode Island's BARL facility, false albacore were held in captivity for more than two years – preceding, cohabitating with, *and* succeeding most other fish (yellowfin and Atlantic bluefin) in the tank – without developing any signs of puffy snout. The capture and transport procedure was similar and the holding conditions for all twelve false albacore were identical to those other tunas experienced. Further investigation into the susceptibility of false albacore to puffy snout is warranted. Based on the findings of the surveys in this report, the

following scombrid species are listed in order of apparent decreasing susceptibility: skipjack and Atlantic mackerel > blackfin > yellowfin > Atlantic bluefin and Pacific bluefin > Pacific mackerel and Pacific bonito > kawakawa > false albacore. An additional two circumstances contribute to the notion of species specificity, though both with regard to puffy snout in non-Scombridae. First, several mahi mahi (*Coryphaena hipparus*) were held at the BARL facility prior to any tunas being held. The capture and transport procedure, holding system, water quality characteristics, and feeding regime were identical or nearly identical to those employed for holding tunas, yet none of the mahi displayed clinical signs of puffy snout development at any time during their nearly-one year in captivity. Capture-induced injuries, tank wall collision-related injuries, and bacterial infections were present, but at no time did these present signs similar to those observed in puffy snout afflictions. This information supports the notion that puffy snout is specific to scombrid fishes. Second, however, is the observation of a barracuda (*Sphyraena* sp.) at a public aquarium displaying clinical signs highly similar to those observed in the early stages of puffy snout. While further investigation was not pursued and thus, the presence of the condition cannot be verified, the observer is a person familiar with the clinical signs of puffy snout and a photograph of the barracuda was taken; examination of the photograph corroborates the observer's suspicion. In contradiction to what has been assumed and supported by a small but detailed body of data, this information suggests that puffy snout may not be exclusive to scombrids. The occurrence of puffy snout in other pelagic fishes should be considered, and efforts to document such potentiality should be undertaken.

There is some evidence that a minimum size threshold exists within species. Two facilities – rearing blackfin and kawakawa, respectively – indicating any difference in size between afflicted and non-afflicted fish stated there was a minimum threshold size. It is unclear, however, if fish exceeded these thresholds while in captivity and developed puffy snout. This information would be valuable in determining whether or not a minimum size threshold exists for puffy snout susceptibility. Given limited data, however, further evidence is needed to validate this notion.

Only one report specified the sex ratio of puffy snout afflicted fish, and it was determined to be equal numbers of males and females. Given the limited data available, it remains unclear if susceptibility is sex-related. Similarly, insufficient evidence exists to determine if progeny of captive broodstock are less likely to develop puffy snout. The facilities that raised progeny of these fish did so only to larval or post-juvenile size, which is likely under a potential size threshold. However, evidence from previous investigations found that domestic fish are less disturbed by potential stressors than their wild conspecifics (Huntingford, 2004; Overli et al., 2005), and that this trait is heritable (Pottinger et al., 1992, 1994; Fevolden et al., 1999; Overli et al., 2005). Fish held with heterospecific tunas were no more or less likely to develop puffy snout. All three facilities rearing more than one species in a common tank reported positive occurrences of puffy snout, but prevalence was highly variable and similar to facilities holding single species, suggesting holding heterospecifics together does not influence the development or rate of prevalence of puffy snout. The time until puffy snout developed also did not appear linked to any factor examined with similar intervals across reports for all species.

Finally, assessing whether puffy snout might be horizontally transmissible was considered one of the more important aspects of the study. Only two facilities indicated that all fish in a rearing unit developed puffy snout, and there was a high degree of variation in prevalence rates among all other facilities reporting puffy snout. These data, coupled with those discussed in section 4.1.3, suggest that if puffy snout is related to a pathogenic agent, it does not appear to be readily transmissible and horizontal transmission is unlikely. Further research in this area is warranted.

A determining factor in whether a fish will develop puffy snout is its wild or captive status; puffy snout is concluded to be solely a captivity-related condition (Table 11). In addition to 100% of the facilities answering the survey having never seen puffy snout in wild fish of any species, one researcher working with Atlantic bluefin tuna handled at least 2,000 adults and several hundred juveniles without once observing it (M. Lutcavage, personal communication).

4.2.2 Capture and Transport

Both capture and transport are well-documented as significant and sometimes lethal sources of stress in fishes (Love, 1970; Mazeaud et al., 1977; Harrell, 1992; Frisch & Anderson, 2000; Cooke et al., 2008; Guindon, 2010; Brooks et al., 2012), including tunas (Barrett & Connor, 1962; Bourke et al., 1987; Skomal and Chase, 1997; Skomal, 2007). In the current report, several aspects of the capture and transport influencing stress [i.e., capture method (Harrell, 1992), biomass density (Gomes et al., 2003)] were investigated. Overall, capture and transport does not seem to be a decisive process in the development of puffy snout (Table 12). It might be

expected that fish which experienced a more stressful transport would subsequently be more likely to develop puffy snout. It could be hypothesised that employing a purse seine capture method, wherein fish are crowded lightly and not directly handled, would impart less stress on a fish, and be less likely to induce puffy snout than traditional hook-and-line capture. Indeed, neither facility employing collection of Atlantic bluefin by purse seine observed puffy snout. Of note, collection by angling by other facilities did not always result in puffy snout. In some cases, fish subjected to more stressful capture and transport processes actually had a lower prevalence. For example, two facilities transporting approximately the same size yellowfin utilizing different transport processes had differing rates of puffy snout prevalence. Contrary to what might be expected, the facility with lower prevalence (20% versus 100%) utilised smaller transport tanks (8-11 m³ versus 18 m³), transported more individuals per tank (6-8 versus 1-4), which together resulted in a higher biomass density (5-10 kg/m³ versus 0.6-4 kg/m³), and had longer transit time to the rearing facility (1-5 days versus 14-30 hours), than the other facility. In addition, puffy snout was typically not noticeable for at least one month after fish are in captivity – seemingly too long of a latency period for a capture/transport-induced mechanism. Previous investigations indicate that the capture/transport procedure is an acute stressor and the resultant physiological responses return to normal after a few hours or days (Barrett and Connor, 1964; Bourke et al., 1987; Arthur et al., 1992; Davidson et al., 1997; Frisch and Anderson, 2000; Chandroo et al., 2005).

4.2.3 Rearing System Design and Water Quality

While the shape of a rearing unit did not influence the development of puffy snout, it remains unclear whether rearing system type (i.e., open-water or land-based), volume, or rearing biomass density do (Table 13). All three open-water holding systems were reported to be absent of puffy snout, but so too were four of the 19 (21%) land-based systems. The differences between open-water and land-based captivity are numerous and complex, and many were outside the scope of this study. A more comprehensive investigation into the role of the type of rearing system in puffy snout development is warranted.

Previous findings suggest that the primary cause of puffy snout was related to confinement (Dizon and Sharp, 1978; Nakamura, 1972). Confinement has at least two distinct parameters: the relationship between the size of a *single* fish and the volume of the tank in which it swims (i.e., rearing unit size), and second, the total biomass density. For example, consider one fish of a given size to be swimming in a tank of a given size. The addition of another fish of the same size to the tank doubles the biomass density but the actual space each fish has to swim in has essentially remained the same. Both fish do not inhabit the exact same space at the same time, and therefore the addition of a second fish *does* reduce the space available for the other, but proportionately less so relative to the increase in biomass density. Now, consider a single fish swimming in that same tank and a divider introduced across the middle. Not only is the biomass density doubled, as it was with the addition of a second fish, but *also* is the space available for that single fish to swim cut in half. It is recognised that these two parameters of confinement approach each other with the continual

addition of fish in an un-divided tank, but for the purposes of this study, they are considered independent.

Space confinement has been demonstrated to induce stress in fish (Dizon et al., 1974; Davidson et al., 1997). The findings of the current report support these previous observations. For example, one facility holding yellowfin reported 20% prevalence in one tank but 80% prevalence in another. The fish in each tank were caught from the same area, subjected to the same transport procedure, supplied with the same seawater, and fed the same food, but were held in different size tanks; the larger tank had a lower puffy snout prevalence than the smaller tank. Additionally, only one of thirteen holding units (8%) larger than 80 m³ was accompanied by a puffy snout prevalence greater than 20%, whereas six of ten holding units (60%) smaller than 80 m³ were accompanied by puffy snout prevalence of greater than 80%. One of the two reports of holding units with volumes of 1,300-1,500 m³ observed puffy snout; just 10% of their yellowfin developed the malady after two or more years in captivity. Finally, neither of the two open-water Atlantic bluefin holding facilities (pen sizes of 49,087 and 58,875 m³, respectively) observed puffy snout. However, much of the remaining data regarding rearing unit size are highly variable and do not suggest a coupling of tank size and puffy snout prevalence. Ultimately, further research is needed to better understand the role of rearing unit size in puffy snout development.

The contribution of biomass density to puffy snout prevalence is also unclear. Considering the volume of evidence that higher densities have been shown to result in higher stress loads with secondary and tertiary consequences (Refstie, 1977; Holm et al., 1990; Montero et al., 1999; Boujard et al., 2002; Ellis, 2002; Ellis et al., 2002;

Vazzana et al., 2002; Gomati et al., 2004; Ashley, 2007), it was hypothesised that puffy snout prevalence would be density-dependant. However, it is not obvious if biomass density alone triggers puffy snout. Both across and within species, reported biomass densities were largely similar though puffy snout prevalence was highly variable. For example, rearing biomass densities in the majority of facilities ranged from 0.3 kg/m³ to 1.6 kg/m³, and puffy snout was absent at some facilities, present in all fish at other facilities, and variable at others. Two facilities with markedly higher densities (2-5 kg/m³) did not observe any puffy snout. When considering smaller-scale differences in holding unit biomass density, particularly within a given species, ascertaining a relationship with puffy snout is an even greater challenge. For instance, when comparing reports in yellowfin of 100% puffy snout prevalence to one with 80% prevalence, the minimum biomass density (1 kg/m³ versus 0.95 kg/m³) at the facilities were similar but maximum biomass densities differed (1.6 kg/m³ versus 0.95 kg/m³). It is therefore plausible that the maximum biomass density that tunas are subjected to is of greater importance in the development of puffy snout. Conversely, when comparing facilities with 80% and 20% puffy snout prevalence, the higher-prevalence report had a higher minimum biomass density (0.95 kg/m³ versus 0.3 kg/m³) but a lower maximum biomass density (0.95 kg/m³ versus 1.5 kg/m³).

Given these contradictory findings, it cannot be definitively concluded that puffy snout is a biomass-density-dependent malady due to an array of complex and dynamic variables. As fish are added and subtracted from a given holding unit, not only does the biomass density change, but so too do other aspects of the environment (i.e., feed addition, waste production) that work in conjunction with one another. It could be

hypothesised that the range of biomass density itself is less predictive of puffy snout emergence, but the duration of a given density within that range that a fish experiences is more likely to play a role. It should be noted that the difficulty in handling live tunas complicates actual weight measurements, and the potential for inaccurate weight estimates is high; this has clear implications for fine-level biomass comparison. Ultimately, more data is needed to better explore this possibility and the contribution of biomass density to puffy snout.

The characteristics of and technologies employed for water treatment examined here were not influential in the development of puffy snout in tunas held in captivity (Table 13). It could have been hypothesised that more selective and aggressive filtration methods would reduce or eliminate the load of pathogens or abiotic materials (i.e., particulates) and therefore result in higher-quality water and lower prevalence of puffy snout. Conversely such filtration might remove or change certain constituents of the water and this resultant ‘un-natural’ water quality could act as a stressor to the fish. Regardless, no coupling was found between any of the water treatment characteristics and the prevalence of puffy snout.

Water quality is one of the most important factors in maintaining fish health. An extensive review of water quality requirements for finfish and the consequences of subpar rearing conditions was conducted by Wedemeyer (1996). In this study, with the exception of unionised ammonia (NH_3) concentration, it was clear that none of the major water quality parameters were responsible for the emergence or development of puffy snout (Table 13). Temperature, dissolved oxygen content, pH, and salinity were within optimal ranges (Farwell, 2003) for all tunas held. Based on metabolic rate,

higher temperatures could have induced more rapid progression of puffy snout, but species-specific temperature preferences and highly variable prevalence within species render this notion unclear at most. Accumulation of toxic or semi-toxic waste products (i.e., nitrogenous waste, respired carbon dioxide) could indeed cause stress. In four reports, NH_3 concentrations exceeded the commonly-accepted threshold of 0.025 mg/L for commercially-cultured finfish (Neori et al., 2004; Chen et al., 2006a; Crab et al., 2007). Three of these reports exceeded this threshold by a mere 0.005 mg/L, while one report for Atlantic bluefin exceeded this threshold by up to 0.075 mg/L, or 360%. While these elevated concentrations could have been a contributor to the emergence of puffy snout, the occurrence of puffy snout at numerous facilities with low levels of ammonia reduce the likelihood of it as significant contributing factor. Ammonia-toxicity is species-specific (Colt, 2004), and a paucity of information regarding the acute and chronic tolerance of tunas to ammonia exposure makes definitive elimination of NH_3 toxicity difficult, but at this time it seems unlikely that NH_3 content was directly responsible for puffy snout. Though CO_2 was not a parameter included in the survey, its low level at the URI BARL facility, where 100% puffy snout prevalence was observed for yellowfin and Atlantic bluefin, reduces the likelihood that it is a determining factor in puffy snout development.

4.2.4 Feeding Regime

Feeding regime was also found not to be a major determinant in the development of puffy snout (Table 14). It was hypothesised that formulated feeds may be used by some facilities and, while nutritional composition of feeds is highly engineered and

thought to approach or equal the value of traditional feed items for several other finfish genera (extensively reviewed by Mourente & Toucher, 2009), this is not the case for scombrids and thus, formulated feed use may contribute to the development of puffy snout. However, no facilities reported using formulated feeds. It was also thought that perhaps natural feed items could have potentially been carrying pathogens (Kim et al., 2007; Slocombe, 2008; Gomez et al., 2010; Kim et al., 2013) or contain enzymes which reduce the bioavailability of certain necessary nutrients (Saunders & Henderson, 1974; Anglesea & Jackson, 1985; Ruohonen et al., 1998; Wistbacka and Bylund, 2008) which might lead to the development of puffy snout. Additionally, vitamin supplementation has been shown to both mitigate (Merchie et al., 1997) and amplify (Dabrowska et al., 1991) the stress response depending on the rate of administration, and the immunosuppressive effects of stress can be moderated by the addition of vitamins to the diet (Jeney et al., 1997; Volpatti et al., 1998). But for all factors examined, none appeared related to the development or prevalence of puffy snout. The feed items used, whether fresh or previously-frozen, the feeding rate, and whether supplementary vitamins were incorporated into the feeding regime had no impact on puffy snout. There are finer-scale nutritional intricacies that are outside the scope of this study which may be involved, and given that inadequate nutrition is known to impact secondary physiological processes such as immune function (Rice, 1990; Wedemeyer, 1996; Ashley, 2007), a poor diet could contribute to or exacerbate a puffy snout affliction. As previously discussed, the development of puffy snout contributes to *and* exacerbates insufficient caloric and nutrient intake. However, whether a newly-captured fish begins feeding or not is also unlikely to play a role in

the emergence of puffy snout. In fact, in some cases, a fish that had not begun feeding in captivity might die as a result of starvation before symptoms of puffy snout began to emerge. Rather, as discussed in section 3.2.1, it is the emergence of puffy snout that plays a role in changes in feeding behaviour.

4.2.5. Survey Participant-Suggested Causes

During the process of preliminary communication with each facility, it was suggested several times that three potential causes of puffy snout were: 1) swelling due to low-impact, blunt wall strikes; 2) scarring due to scraping, abrasive wall strikes, and 3) facial swelling related to capture by hook and line. It seems, however, that none of these suggestions is likely.

None of the reports mentioned, explicitly or implicitly, that wall strikes of any kind were noticeably linked to the emergence or prevalence of puffy snout. Additionally, and more substantially, several hundred hours of fish observation at our own facility, both during transport and during land-based captivity, yielded almost zero incidences of wall strikes. Fish seemed to inherently sense the presence of their bounds, perhaps aided by striping installed on tank walls (Farwell, 2001; Wexler et al., 2003; Ishibashi et al., 2013). It was not until after puffy snout afflictions were established, when vision was greatly impaired, that even occasional wall strikes (albeit very low-impact ‘bumps’) were observed. It therefore seems that swelling due to impact trauma can be disregarded. Whereas deformities of the head and jaws of juveniles, whose bones are incompletely ossified and thus subject to impact-caused malformation, have indeed been linked to wall strikes (Miyashita et al., 2000, Masuma

et al., 2001; Wexler et al., 2003; De Metrio et al., 2010), puffy snout is different in many ways. If wall strikes were frequent or severe enough to cause fractures and subsequent misaligned healing, they would be readily identified and not mistaken as the development of puffy snout.

It was also determined, through subsurface tank-wall windows that no abrasions, which would cause the suggested scarring, were observed on the head. If abrasions were seen, they were exclusively posterior to the head, either in the area of the pectoral fin or on the lateral keels of the caudal peduncle. The healing process of those abrasions is distinctive and conspicuous, and at no time was it observed to be occurring on the head. Scarring, therefore, may be omitted as a potential cause of puffy snout.

While there is evidence that an inflammatory response may occur early on the condition's development, and it is plausible that hook-and-line capture induces this inflammation, remaining data suggest that facial swelling due to the pressure of the hook and line used during capture is responsible for puffy snout can most likely be eliminated. Though neither report of purse-seine-captured Atlantic bluefin observed puffy snout, the differences between these operations and the remainder of reports, all but one being land-based, are many. Additionally, the wide range of puffy snout prevalence rates among hook-and-line-captured fish that were subsequently placed in similar captive conditions lessens the probability that hook-and-line capture causes puffy snout.

4.3. *Questions for Further Study*

To more comprehensively understand puffy snout and its effects, more detailed physical and physiological investigations should be undertaken. First, an evaluation of blood chemistry parameters would help to provide evidence for the notion that puffy snout is in fact rooted in stress, and may allow for quantification of that stress. This approach is common among fish researchers (Wood, 1991; Frisch & Anderson, 2000; Barton, 2002; Ashley, 2007; Frick et al., 2009; Pankhurst, 2011). To establish blood chemistry values indicative of stress, a baseline of ‘resting’ values must also be established, and obtaining serial blood samples from tuna is, in many cases, a prohibitively difficult task. Furthermore, certain constituents are less indicative of chronic stress (i.e., epinephrine) (Iwama, 1998; Barton, 2002) and also more likely to be elevated during the process of obtaining the blood sample, so care must be taken when choosing the constituents and evaluating their values. However, if a process which adequately addresses those issues can be devised, a better understanding of the stress load a captive tuna carries can be attained. To understand how stress might specifically affect the development and progression of puffy snout, blood chemistry parameters should be compared between wild fish, captive fish showing no signs of puffy snout, and captive fish exhibiting puffy snout.

To translate the stress load a captive tuna experiences into more tertiary metrics, investigations into growth, immune function, and sexual maturation and reproductive activity should be employed. All have been shown to be compromised by chronic stress in other species of fish (see Barton et al., 1987; Pickering et al., 1991; Pickering,

1992; Balm, 1997; Pankhurst & Van der Kraak, 1997; Contreras-Sanchez et al., 1998; McCormick, 1998, 1999; Einarsdottir et al., 2000; Schreck et al., 2001; Ashley, 2007). With respect to the correlation between growth rate and presence of puffy snout, serial measurements – most likely length – could be employed and compared between groups. If blood was to be drawn so some of its constituents were used for the evaluation of stress load, so too could some of its constituents be used to evaluate reproductive status (e.g., luteinizing hormone, follicle stimulating hormone) (Schreck et al., 2001; Schreck, 2010) and immune function or response of specific (e.g., antibodies, T lymphocytes) (Kaattari & Piganelli, 1997; Manning & Nakanishi, 1997) or nonspecific origin (e.g., macrophages, phagocytes, lysozymes) (Secombes, 1997; Yano, 1997) (Schreck, 1997; Tort, 2011). The challenges associated with these activities, however, are presently prohibitive and must be carefully considered before employment.

One major area not investigated here but worthy of future consideration is the sound and vibration inside the tank. Pumps, air diffusers, and other aquaculture equipment may transfer both noise and vibration energy into the water, and in an enclosed tank, that energy has limited opportunity to dissipate (Bart, et al., 2001; Davidson et al., 2007; Wysocki et al., 2007). The in-tank environment may be analogous to a bell; while a rung bell may be loud for someone standing next to it, the energy *inside* is amplified and would elicit a much different response from someone experiencing the ring from within. It has been demonstrated that aquaculture production noise is within the audible range for most fish species (Bart et al., 2001), and despite an idea that random sounds would be more stressful than chronic ones

because of their unpredictability (Craven et al., 2009), chronic noise and/or vibration energy might serve as an irritant to the fish, perhaps over-stimulating components of the central nervous system (i.e., otolith organs, lateral line organ), and result in a reactive physiological response. The merit of this notion may be supported by the fact that none of the fish held in open-water systems became afflicted with puffy snout. Indeed, some fish held in land-based systems also never developed puffy snout, but the sound- and vibration-producing specifics of the holding units and their life support systems may have been less severe than those systems in which fish which did develop puffy snout (e.g., a concrete tank may be less amplifying than a fiberglass tank, or a pump located a given distance away from the tank may result in a lower energy transference), or, as is seemingly the case, some fish may be more or less tolerant of the puffy snout-inducing factor(s) (e.g., skipjack seem to be more susceptible to puffy snout than false albacore). To further examine the possibility of sound and vibration energy contributing to the development of puffy snout, measurements of that energy could be taken using a hydrophone or a particle motion sensor and comparisons made between facilities' sound profiles and the occurrence of puffy snout.

An additional condition not explored here but of potential interest is exposure of the fish to natural light, photoperiod, and lunar cycle. These factors are known to serve as cues for biological activities, namely spawning (Schaefer, 2001; Medina et al., 2002; Corriero et al., 2003; Abascal et al., 2004; Medina et al., 2007) and captivity has been shown to induce stress which impedes the normal function of such activities (Mylonas & Zohar, 2001, 2007; Zohar & Mylonas, 2001; Lambert & Thorsen, 2003;

Mylonas et al., 2010; Aranda et al., 2011). It is not, therefore, far-fetched to consider that land-based captivity, where there is often artificial simulation of natural phenomena, would cause other physiological or endocrinological dysfunction – including immuno-compromisation – which could either result in or not combat puffy snout. None of the reports of open-water holding, where fish experience natural light, photoperiod, and lunar cycle conditions, observed puffy snout, but some land-based facilities which also are designed such that fish experience such natural conditions did observe puffy snout (e.g. Kewalo Basin Research Lab) – some of which at just-as-high prevalence rates as fully-indoor facilities (e.g., URI BARL). At this time, it seems unlikely that a fish's exposure to natural conditions or artificial replications of those conditions contributed to the development of puffy snout, but future research into the effect of such simulation is warranted.

Finally, a limiting factor in this study was the number of responding facilities. Indeed, confidence in the data received – albeit limited – is high, but the conclusions drawn here are done so with that reservation, and future work would benefit greatly from a more numerous and diverse group of facilities.

5. Conclusion

Puffy snout syndrome is a condition characterised by tumour-like growths which first appear on the dorsal surface of the head and the snout of tunas, and move ventrally and posteriorly as the condition progresses. These lesions will occlude the eyes and the mouth, and likely the nares, of an afflicted fish, and interfere with feeding and swimming behaviours. If allowed to progress, mortality will result, most likely due to lack of food as a result of inability to see and/or consume feed items or an exhaustion of physiological resources to cope with the stress of puffy snout itself and/or the conditions that caused it. Though few mentions of puffy snout can be found in the literature, personal and anecdotal evidence suggested that puffy snout is relatively common among facilities that hold tunas in captivity. It was the aim of this study to a) describe pathological features of puffy snout, and b) determine its etiology through the evaluation of conditions and/or processes that tuna-holding facilities employ.

Broad-scope parasitology, bacteriology, and virology all returned results suggesting that no commonly-found pathological agents are responsible for inducing puffy snout. However, given that puffy snout is an uncommon condition (i.e. potentially exclusive to scombrids), it is quite possible that an uncommon pathological agent is, at least in part, partially responsible.

The histological evidence suggests that puffy snout may be the result of a progression from chronic inflammation to fibrosis. Certain elements of each condition were observed in the tissue but not all elements of either condition were present. It is

clear, however, that vascularised muscle tissue degenerates and is replaced in space with a loose, poorly-organised collagen matrix. The cellular landscape does not support the presence of an active inflammatory response, but the tissues examined in this study may have been excised from fish beyond the inflammatory stage and in the fibrotic stage. Further research is warranted to better identify and quantify the cells present at various stages of puffy snout progression. Such work would be beneficial in beginning to understand how the condition is initiated and perpetuated.

The capture and transport procedure and the feeding regime are not predictive measures of puffy snout development, but some features of tuna biology and the rearing system they are held in may be. The findings of this report suggest that susceptibility to puffy snout is species-specific, and there may be a minimum size threshold for susceptibility within each species. The size of the rearing unit appears a more significant factor than the biomass density inside that unit. Importantly, this study eliminated many factors hypothesised to be potential role-players in puffy snout emergence and/or development, but more research is needed in the areas not definitively eliminated (Table 15). The only factor investigated in this study found to be a definitive predictor of puffy snout development in tunas is whether a fish is wild or has been transported from the wild and is reared in captivity: puffy snout is solely a captivity-related condition.

As the interest in holding tunas in captivity grows, understanding puffy snout will become more important. For those facilities that aim to conduct physiological research, captivity allows consistent access to an otherwise-migratory group of species whose biological and ecological characteristics make them difficult to study. For

public display facilities, the ability to hold tunas allows them to showcase and share one of the world's most iconic fish with citizens who otherwise would have little or no opportunity to see them. For aquaculture operations, the ability to hold broodstock, close the life cycle, and produce egg-to-plate tuna can not only bring a financially valuable product to market, but also help to reduce pressure on wild stocks by contributing to that market's demand. It is my hope that this work will be of use to each of those parties, and contributes in some way to understanding such majestic and magnificent fish.

Table 1. Ten fish were used for the investigation of puffy snout pathology. Eight were held in captivity prior to death or euthanasia and two were euthanized at sea immediately-post capture.

Specimens Used for Investigation of Puffy Snout Pathology			
Species	Group	Date Captured	Date Necropsied
<i>Thunnus thynnus</i>	Captive	30 August 2012	7 February 2013
<i>Thunnus thynnus</i>	Captive	31 August 2012	7 February 2013
<i>Thunnus albacares</i>	Captive	7 August 2013	12 September 2013
<i>Thunnus albacares</i>	Captive	17 July 2013	16 September 2013
<i>Thunnus albacares</i>	Captive	7 October 2012	1 August 2013
<i>Thunnus albacares</i>	Captive	17 July 2013	1 November 2013
<i>Thunnus albacares</i>	Captive	21 September 2013	17 June 2014
<i>Euthynnus alleteratus</i>	Captive	September 2012	22 April 2014
<i>Thunnus albacares</i>	Wild	5 September 2014	5 September 2014
<i>Thunnus albacares</i>	Wild	5 September 2014	5 September 2014

Table 2. The size range of fish which did and which did not develop puffy snout syndrome, and their relationship to puffy snout prevalence in ten scombrid species reared in captivity. (N/A: not applicable or not available)

Size Range and Puffy Snout Prevalence			
Species	PS Prevalence	Size w/ PS	Size w/out PS
<i>T. albacares</i>	0%	N/A	68-200 cm / 58-168 kg
	1%	50 cm / 3 kg	20-155 cm / 0.5-70 kg
	10%	N/A	N/A
	20%	50-150 cm / 6-60 kg	Same
	20%	40-50 cm / 1-3 kg	Same
	80%	40-50 cm / 1-3 kg	Same
	100%	65-130 cm / 6-40 kg	Same
<i>T. atlanticus</i>	80-90%	50-90 cm / 3- 14.5 kg	<50 cm / 0.19-3 kg
<i>T. orientalis</i>	20%	50-150 cm / 6-60 kg	Same
<i>T. thynnus</i>	0%	N/A	
	0%	N/A	3-100 g, 50-400 kg
	0%	N/A	<15 cm
	100%	130 cm / 45 kg	N/A
	100%	70-80 cm / 8-11 kg	N/A
<i>E. affinus</i>	0%	N/A	40-50 cm / 1-3 kg
	10%	50-65 cm / 1-5 kg	10-15 cm / <1 kg
<i>E. alleteratus</i>	0%	N/A	40-60 cm / 2-4 kg
	0%	N/A	60 cm / 4-5 kg
<i>K. pelamis</i>	100%	40-50 cm / 1-3 kg	N/A
<i>S. chiliensis</i>	20%	15-35 cm	Same
<i>S. japonicus</i>	20%	15-35 cm	Same
<i>S. scombrus</i>	100%	45 cm	Same

Table 3. The duration of time between fish introduced to the captive rearing unit and the first clinical signs of puffy snout syndrome, and its relationship to puffy snout prevalence in seven scombrid species reared in captivity (N/A: not applicable)

Time Until Development and Puffy Snout Prevalence		
Species	PS Prevalence	Time Until First Observed
<i>T. albacares</i>	0%	N/A
	1%	2 months
	10%	> 2 years
	20%	1-6 months
	20%	1-4 months
	80%	1-4 months
	100%	3 weeks – 2 months
<i>T. atlanticus</i>	80-90%	1 month
<i>T. orientalis</i>	20%	1-6 months
<i>T. thynnus</i>	0%	N/A
	0%	N/A
	0%	N/A
	100%	10-12 months
	100%	2 months
<i>E. affinus</i>	0%	N/A
	10%	1-6 months
<i>E. alleteratus</i>	0%	N/A
	0%	N/A
<i>K. pelamis</i>	100%	2-3 weeks

Table 4. The method of capture, number of times handled between capture and placement in captivity, duration of transport, and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not applicable or not available. * denotes a direct quote from survey respondent)

Capture & Transport Variables and Puffy Snout Prevalence				
Species	PS Prevalence	Capture Method	Times Handled	Transport Duration
<i>T. albacares</i>	0%	Handline	3-4	1 – 3 hours
	1%	Rod & reel	1-2	0.75 – 3 hours
	10%	Rod & reel, Handline	2-3	1 hour
	20%	Rod & reel, Liftpole	3	1 – 5 days
	20%	Rod & reel	1-2	1 – 3 hours
	80%	Rod & reel	1-2	1 – 3 hours
	100%	Rod & reel	1	14 – 30 hours
<i>T. atlanticus</i>	80-90%	Rod & reel	1-2	1 – 3 hours
<i>T. orientalis</i>	20%	Rod & reel, Liftpole	3	1 – 5 hours
<i>T. thynnus</i>	0%	Purse seine	0	“Several days”*
	0%	Purse seine	0	5 – 10 days
	0%	N/A	N/A	N/A
	100%	Rod & reel	1	3 – 6 hours
	100%	Rod & reel	1-2	2 days – 3 weeks
<i>E. affinus</i>	0%	Rod & reel	1-2	1 – 3 hours
	10%	Rod & reel, Liftpole	3	1 – 5 days
<i>E. alleteratus</i>	0%	Rod & reel	1	3 – 6 hours
	0%	Rod & reel	1-2	2 days – 1 month
<i>K. pelamis</i>	100%	Rod & reel, Liftpole	1-2	1 – 3 hours

Table 5. The size and shape of the tank or pen used for transport from the wild to the site of captive rearing, and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not applicable or not available)

Transport Unit Size & Shape and Puffy Snout Prevalence			
Species	PS Prevalence	Unit Size (m³)	Unit Shape
<i>T. albacares</i>	0%	2	Oval
	1%	0.2, 0.6	Round, rectangular
	10%	1.4, 1.7	Rectangular
	20%	8, 11.4	Rectangular
	20%	6	Oval & rectangular
	80%	6	Oval & rectangular
	100%	18, 18.5	Cube & rectangular
<i>T. atlanticus</i>	80-90%	1.13, N/A	Round, “Tuna Tube”
<i>T. orientalis</i>	20%	8, 11.4	Rectangular
<i>T. thynnus</i>	0%	49,087	Round
	0%	58,874	Round
	0%	N/A	N/A
	100%	2	Round
	100%	1.1, 3.35	Oval
<i>E. affinus</i>	0%	6	Oval & rectangular
	10%	8, 11.4	Rectangular
<i>E. alleteratus</i>	0%	2	Round
	0%	1.1, 3.35	Oval
<i>K. pelamis</i>	100%	8, 11.4	Oval & rectangular

Table 6. The size of fish at transport, the number of fish transported per trip, the biomass density inside the transport unit, and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not applicable or not available)

Fish Transport Variables and Puffy Snout Prevalence				
Species	PS Prevalence	No. of Fish/Trip	Fish Size (kg)	Biomass Density (kg/m³)
<i>T. albacares</i>	0%	1	1 – 3 (max 10)	3-4
	1%	3-6	1 – 3	1-2
	10%	1-3	1.8-10 (2-5 typical)	2-3
	20%	6-8	7 – 19	3
	20%	10-20	0.5 – 3.6	1-2
	80%	10-20	0.5 – 3.6	1-2
	100%	1-4	8 – 25	1
<i>T. atlanticus</i>	80-90%	2-4	0.19 – 14	1-2
<i>T. orientalis</i>	20%	6-8	7 – 15	3
<i>T. thynnus</i>	0%	N/A	N/A	0
	0%	400-600	250	0
	0%	N/A	N/A	N/A
	100%	1-2	7 – 10	1
	100%	2-3	10 – 12	1-2
<i>E. affinus</i>	0%	10-20	1-2	1-2
	10%	N/A	3	3
<i>E. alleteratus</i>	0%	7-10	1	1
	0%	N/A	1-2	1-2
<i>K. pelamis</i>	100%	10-20	1-2	1-2

Table 7. The volume of the rearing unit, the biomass density inside it, and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not available)

Rearing Unit Size, Biomass Density and Puffy Snout Prevalence			
Species	PS Prevalence	Rearing Unit Size (m³)	Biomass Density (kg/m³)
<i>T. albacares</i>	0%	1,500 (235 acclimation)	0.5 – 1.0
	1%	7 – 250	0.3 – 7.0
	10%	1,360	0.5
	20%	110, 330	1.0 – 1.5
	20%	66	0.3 – 1.5
	80%	46	0.95
	100%	75	1.0 – 1.6
<i>T. atlanticus</i>	80-90%	80	0.25 – 1.0
<i>T. orientalis</i>	20%	110, 330	1.0 – 1.5
<i>T. thynnus</i>	0%	49,087	2.0 – 5.0
	0%	58,875	0.05, 2.19
	0%	N/A	N/A
	100%	75	N/A
	100%	305	0.3 – 1.5
<i>E. affinus</i>	0%	46, 66	0.37 – 1.5
	10%	110, 330	1.0 – 1.5
<i>E. alleteratus</i>	0%	75	0.5 – 1.6
	0%	83	N/A
<i>K. pelamis</i>	100%	46, 66	0.37 – 1.5

Table 8. Characteristics of rearing system design and water treatment and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not applicable or not available)

Rearing Unit Water Treatment and Puffy Snout Prevalence						
Species	PS Prev.	Percent Recirc.	Turnovers/ Day	Biofiltration	µm Size Removed	UV
<i>T. albacares</i>	0%	50	2-3	Yes	N/A	No
	1%	N/A	N/A	Yes	N/A	Yes
	10%	95	7-8	Yes	20	Yes
	20%	100	12	Yes	N/A	Yes
	20%	0	10	N/A	N/A	No
	80%	0	10	N/A	N/A	No
	100%	60-70	10-11	Yes	5	Yes
<i>T. atlanticus</i>	80-90%	70-90	3.5	Yes	20	Yes
<i>T. orientalis</i>	20%	100	12	Yes	N/A	Yes
<i>T. thynnus</i>	0%	0	N/A	N/A	N/A	No
	0%	0	N/A	N/A	N/A	No
	0%	N/A	N/A	N/A	N/A	N/A
	100%	60-70	10-11	No	50	Yes
	100%	N/A	N/A	N/A	N/A	No
<i>E. affinus</i>	0%	0	10	No	N/A	No
	10%	100	12	Yes	N/A	Yes
<i>E. alleteratus</i>	0%	60-70	10-11	No, Yes	50, 5	Yes
	0%	0	N/A	N/A	N/A	N/A
<i>K. pelamis</i>	100%	0	10	N/A	N/A	No

Table 9. Water quality parameters inside rearing units and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (N/A: not available)

Rearing Unit Water Quality and Puffy Snout Prevalence						
Species	PS Prev.	°C	O ₂ Saturation	pH	NH ₃ (mg/L)	Salinity (ppt)
<i>T. albacares</i>	0%	27-29	85-100%	7.9 – 8.3	N/A	32-34
	1%	24-26	90%	8.1	“ ~0 ”	34
	10%	20-30	65-107%	7.6 – 8.3	N/A	26-31
	20%	20	98-100%	7.8 – 7.9	≤ 0.03	N/A
	20%	23-25	100%	7.6	N/A	33
	80%	23-25	100%	7.6	N/A	33
	100%	18-24	80-150%	7.3 – 8.2	≤ 0.004	28-33
<i>T. atlanticus</i>	80-90%	20-30	100-250%	7.8	< 0.02	30-36
<i>T. orientalis</i>	20%	20	98-100%	7.8 – 7.9	≤ 0.03	N/A
<i>T. thynnus</i>	0%	13.5-28	100%	8.0	N/A	38
	0%	22-25	100%	7.8 – 8.1	N/A	36
	0%	N/A	N/A	N/A	N/A	N/A
	100%	18-24	80-150%	7.3 – 8.2	0.004 – 0.1	28-33
	100%	15-27	N/A	7.8 – 8.0	< 0.09	32
<i>E. affinus</i>	0%	23-25	100%	7.6	N/A	33
	10%	20	98-100%	7.8 – 7.9	≤ 0.03	N/A
<i>E. alleteratus</i>	0%	18-24	80-150%	7.3 – 8.2	0.004 – 1	28-33
	0%	22-24	100%	N/A	N/A	N/A
<i>K. pelamis</i>	100%	23-25	100%	7.6	N/A	33

Table 10. Feeding regime and their relationship to puffy snout syndrome prevalence in seven scombrid species reared in captivity. (**A**: anchovy; **BF**: butterfish; **H**: herring; **M**: mackerel; **Sa**: sardine; **Sq**: squid; **N/A**: not available)

Feeding Regime Variables and Puffy Snout Prevalence				
Species	PS Prevalence	Feed Items	Feed Rate	Vitamins Added
<i>T. albacares</i>	0%	Fresh; M, Sq	5-10% BW/d	C, E, Complex daily
	1%	Frozen; Baitfish	N/A	Complex @ 1% feed, C @ 500 mg/fish/d
	10%	Frozen; Sq, H, A	N/A	Mazuri®
	20%	Sq, Sa	32 kcal/kg/d	No
	20%	N/A	Satiation	No
	80%	N/A	Satiation	N/A
	100%	Frozen; Sq, H, A, BF	1-5% BW/d	Mazuri®, SeaTabs®
<i>T. atlanticus</i>	80-90%	N/A	4-12% BW/d	Weekly
<i>T. orientalis</i>	20%	Sq, Sa	32 kcal/kg/d	Mazuri®
<i>T. thynnus</i>	0%	N/A	6 days/week	Complex
	0%	Baitfish	Satiation	No
	0%	N/A	N/A	N/A
	100%	Frozen; S, H, BF	1-5% BW/d	SeaTabs
	100%	H, Sq, M	N/A	N/A
<i>E. affinus</i>	0%	N/A	Satiation	No
	10%	Sq, Sa	32 kcal/kg/d	Mazuri®
<i>E. alleteratus</i>	0%	Frozen; Sq, H, BF, A	1-5% BW/d	Mazuri®, SeaTabs®
	0%	H, Sq, M	N/A	N/A
<i>K. pelamis</i>	100%	10-20	Satiation	No

Table 11. Of eight independent biological characteristics examined, only one was a definitively-determining factor in the emergence of puffy snout syndrome in tunas. Two were found to not play a role. Further examination is needed to draw concrete conclusions for five characteristics.

Determining Factors in the Emergence of Puffy Snout			
BIOLOGICAL	YES	NO	TBD
Species			✓
Size			✓
Sex			✓
Heterospecific Cohabitation		✓	
Transmissible			✓
Captive vs. Wild	✓		
Captive Progeny			✓
Time to Development		✓	
TOTAL	1	2	5

Table 12. All eight examined characteristics of the capture and transport procedure were found to not contribute to the emergence of puffy snout syndrome in tunas.

Determining Factors in the Emergence of Puffy Snout			
CAPTURE & TRANSPORT	YES	NO	TBD
Capture Method		✓	
Times Handled		✓	
Transport Unit Volume		✓	
Transport Unit Shape		✓	
Fish Transported per Trip		✓	
Fish Size at Transport		✓	
Transport Biomass Density		✓	
Transport Time		✓	
TOTAL	0	8	0

Table 13. Eleven characteristics of the holding system design and environment were found to not contribute to the emergence of puffy snout syndrome in tunas. The contribution of three additional characteristics is unclear..

Determining Factors in the Emergence of Puffy Snout			
HOLDING SYSTEM	YES	NO	TBD
System Type			✓
Holding Unit Volume			✓
Holding Unit Shape		✓	
Holding Biomass Density			✓
Degree of Water Re-Use		✓	
Degree of Total Water Turnover		✓	
Use of Biofiltration		✓	
Selectivity of Mechanical Filtration		✓	
Influent Water UV Treatment		✓	
Water Temperature		✓	
Water Oxygen Content		✓	
Water pH		✓	
Water Unionised Ammonia		✓	
Water Salinity		✓	
TOTAL	0	11	3

Table 14. All six characteristics of the feeding regime were found to not contribute to the emergence of puffy snout syndrome in tunas.

Determining Factors in the Emergence of Puffy Snout			
FEEDING	YES	NO	TBD
Percent of Fish to Feed in Captivity		✓	
Time to First Feeding		✓	
Feed Items		✓	
Fresh or Previously-Frozen Feed Items		✓	
Feeding Rate		✓	
Vitamin Supplementation		✓	
TOTAL	0	6	0

Table 15. Four biological characteristics and two features of the holding system design and environment may contribute positively or negatively to puffy snout emergence, but there is currently insufficient data to draw concrete conclusions.

Determining Factors in the Emergence of Puffy Snout			
TOTALS	YES	NO	TBD
Biological Data	1	3	4
Capture & Transport Data	0	8	0
Holding System Data	0	12	2
Feeding Data	0	6	0
TOTAL	1	29	6

Figure 1: The progression of the clinical signs of puffy snout syndrome on the head region of yellowfin tuna (*Thunnus albacares*). Each inset is a different fish. The fish in inset *a* is a wild group fish, absent of any signs of puffy snout, and the severity of the condition increases from inset *b* through inset *f*. The yellow arrow in inset *f* points to the occluded eye.

Figure 2: A yellowfin tuna (*Thunnus albacares*) exhibiting markedly-progressed puffy snout syndrome.

Figure 3: An Atlantic bluefin tuna (*Thunnus thynnus*) exhibiting severe, late-stage puffy snout syndrome.

Figure 4: Tissue immediately anterior to the eye in two yellowfin tuna [(a) and (b)] and one Atlantic bluefin tuna (c) shown in cross section and stained with hematoxylin and eosin (H&E). (a) Tissue from a wild fish absent of any signs of puffy snout. (b) and (c) Tissue from two fish which developed puffy snout.

Figure 5: Tissue immediately anterior to the eye in three yellowfin tuna shown in cross section and stained with H&E. **(a)** A wild fish with no signs of puffy snout syndrome. **(b)** A fish which developed puffy snout and died after 62 days in captivity. **(c)** A fish which developed puffy snout and died after 299 days in captivity.

Figure 6: Tissue immediately anterior to the eye in two yellowfin tuna with puffy snout syndrome shown in cross section. **(a)** Tissue stained with H&E. **(b)** Tissue stained with Masson's trichrome.

Figure 7: Sub-epidermal tissue immediately anterior to the eye in three tunas shown in cross section and stained with H&E. **(b)** Wild yellowfin tuna absent of any signs of puffy snout. **(a)** and **(c)** Two yellowfin tuna afflicted with puffy snout.

Figure 8: Tissue immediately anterior to the eye in four yellowfin tuna shown in cross section and stained with Masson's trichrome. **(a)** and **(c)** Tissue from two wild fish without signs of puffy snout. **(b)** and **(d)** Tissue from two fish which were held in captivity and developed puffy snout. Tissues shown in **(a)** and **(b)** were identical in anatomical location. Tissues shown in **(b)** and **(d)** were in identical anatomical location.

Figure 9: Tissue immediately anterior to the eye in two yellowfin tuna, shown in cross section and stained with Masson's trichrome. **(a)** Tissue from a wild fish absent of any signs of puffy snout. **(b)** Tissue from a fish which was held in captivity and developed puffy snout.

Figure 10: Dorsal musculature tissue, between the first and second dorsal fins, in two yellowfin tuna, shown in cross section and stained with Masson's trichrome. **(a)** Tissue from a fish absent of any signs of puffy snout. **(b)** Tissue from a fish which was held in captivity and developed puffy snout.

Figure 11: Dorsal musculature tissue in two yellowfin tuna, shown in cross section and stained with Masson's trichrome. **(a)** Tissue from between the first and second dorsal fins of a fish with moderately-progressed puffy snout. This tissue was relatively more normal in clinical appearance. **(b)** Tissue from the leading edge of the first dorsal fin of a fish with marginally-progressed puffy snout. This tissue was more abnormal in clinical appearance.

Figure 12: Tissue from captive false albacore, which did not develop puffy snout. **(a)** Tissue taken from anterior to the eye. **(b)** Dorsal musculature tissue.

APPENDIX A: SURVEY, INTRODUCTORY LETTER, PHOTO-COLLAGES OF PUFFY SNOUT AFFLICTIONS

Puffy Snout Inquiry

Taylor Voorhees

University of Rhode Island

+1 609 577 2873

taylorvoorhees@my.uri.edu

Name: _____

Institution: _____

At this facility, which of the following species have been:

*Please check all boxes that apply

	Held	Estimated size range (FL or Wt) of PS-afflicted fish	Estimated size range (FL or Wt) of non-PS- afflicted fish	Estimated Percentage w/ PS
Thunnus				
albacares				%
atlanticus				%
maccoyii				%
obesus				%
orientalis				%
thynnus				%
Katsuwonus				
pelamis				%
Euthynnus				
affinus				%
alletteratus				%
lineatus				%
Other spp.				
				%
				%
				%

Fish Data

What is the typical total biomass density in each rearing unit (kg/m³)?

If sexing PS-afflicted fish was done, what was the male:female ratio? _____:_____ N/A

Were there fish that never became afflicted with PS reared with PS-afflicted fish?

YES / NO

Were there ever multiple species held in the same tank? YES / NO

If so, which species? _____

How much time typically elapsed between a fish's introduction to its captive environment and its noticeable affliction with PS? _____

If applicable, was PS seen in fish that were spawned in captivity? YES / NO / N/A

Have you ever observed wild fish afflicted with PS? YES / NO

If so, which species, how many individuals, their size and their location

Are there any other observations or details regarding captive fish you wish to share or comment on?

Wild Capture and Transport Procedures

What fishing methods were used for capture of wild fish (i.e. rod and reel, seining, etc.)?

If selective fishing methods were used (i.e. rod and reel), what factors determined which fish were transported and which were not (i.e. fish size, fight time, foul-hooking, etc.)?

Please briefly describe the transport units used (i.e. in-deck tank, on-deck tank, square, circular, flume design, floating sea cage, etc.), and their volume.

What was the target biomass density during transport? _____

What was the target number of individual fish to be transported per trip? _____

How much time typically elapsed between a fish's capture and its transfer to its final holding unit?

How many times were fish handled *between* their capture and their transfer to final holding unit?

Are there any details relative to fish transport that you wish to comment on?

Holding System

The holding units at your facility are: Land-based / Net-pen / Both

Shape, volume and dimensions of holding unit(s):

Single-pass Recirculating - If recirculating, to what degree? _____%

Estimated tank turnovers per day _____

If there is a recirculating component, is biofiltration included? YES / NO

What is the finest particle size removed from influent water by mechanical filtration?

_____ Is there UV treatment for influent water? YES / NO

Are there any comments or details regarding your rearing system that you wish to comment on?

Water Quality

What is the minimum, maximum and mean of the water parameters below that afflicted fish were exposed to?

Temperature _____/_____/_____

Dissolved oxygen (in mg/L or % saturation) _____ / _____ / _____

pH _____ / _____ / _____

TAN OR NH₃ _____ / _____ / _____

Salinity _____ / _____ / _____

Were non-PS-afflicted fish held in the same conditions? YES / NO

If NO, please provide information below:

Temperature _____ / _____ / _____

Dissolved oxygen (in mg/L or % saturation) _____ / _____ / _____

pH _____ / _____ / _____

TAN OR NH₃ _____ / _____ / _____

Salinity _____ / _____ / _____

Are there any observations regarding water quality that you wish to comment on?

Feeding

What is the estimated percentage of fish that began to feed in captivity? _____ %

What is the estimated percentage of these fish that eventually developed PS? _____ %

Was there a difference in time to first feeding in fish that eventually developed PS?

YES / NO

If so, what was the estimated difference (i.e. days/weeks before or after)? _____

What was the feeding regime (type of feed and feeding rate) for fish held?

What was the condition of PS when feeding behaviour of afflicted fish changed (i.e. prior to visible signs, initial head wrinkling, partial eye/mouth occlusion, etc.)?

In what percentage of PS-afflicted fish was there a complete cessation of feeding? _____ %

Did any PS-afflicted fish that ceased feeding resume feeding? YES / NO

How much time elapsed between cessation and resumption?

Were supplemental vitamins added to feed items? YES / NO

If so, which ones and in what regime (i.e. daily, weekly, etc.)?

Were prophylactic medicines administered? YES / NO

If so, which ones and through which route of administration?

Are there any observations relative to feeds or medicines that you wish to comment on?

Incidence of Remediation

What, if any, active attempts at remediation of PS were enacted?

Which, if any, were successful? _____

Did any fish show signs of PS remediation without active attempts?

YES / NO

Are there any observations germane to this section that you wish to comment on?

May I contact you for further inquiry? If so, please provide most convenient means of contact.

In advance, thank you very much for your cooperation and your time;
both are greatly appreciated. I look forward to sharing the results.

Puffy Snout Inquiry

Taylor Voorhees

University of Rhode Island

+1 609 577 2873

taylorvoorhees@my.uri.edu

Dear ,

My name is Taylor Voorhees, and I am a graduate student at the University of Rhode Island. As a component of my Master's thesis research, I am investigating the occurrence of 'puffy snout,' a condition arising in captive scombrids in which tumour-like growths form on the head region of afflicted fish and, in extreme cases, occlude the eyes and mouth. Potential clinical signs include the disruption of normal swimming and feeding behaviours and abnormal physiological conditions, having consequences for reproduction, secondary disease resistance and other essential life processes. If allowed to progress, eventual mortality may occur. At URI, we have observed puffy snout in both Atlantic bluefin and in yellowfin tuna, but we are aware that this is in no way a solitary phenomenon.

To date, only seven pieces of published literature have made mention of puffy snout; none of which provided detailed descriptions or characterisations of its effects, nor defined its cause. Thus far, it has been deemed merely a "stress-related condition" potentially resulting from confinement. My aim is to describe puffy snout, through both gross and histological examination, and to investigate its etiology through assessment of capture and transport strategies, water quality, tank design, nutrition, and other conditions that are anticipated to cause such stress. Descriptions can be made from examining our own fish, but to truly understand when and why puffy snout occurs, information must be gathered from all those who have (and equally important, those who have not) observed it.

Attached to this e-mail are two PDF files. The first is a collection of photographic images of puffy snout at varying degrees of severity. The second is a short survey I have developed, requesting information in some key areas that I suspect have an influence in the emergence or non-emergence of puffy snout.

It would be deeply appreciated if you could complete the survey. If you are unable to complete it in its entirety, please contribute as much as you can; for this little-studied malady, every piece of information is valuable. In return, I would be happy to share my conclusions with you by providing an electronic copy of my thesis upon its completion and defence.

Your submitted survey will in no way be shared with or reproduced for any person or entity outside myself and my thesis committee. Furthermore, the information you provide will be evaluated independent of your name and your institution.

Thank you in advance for your cooperation. Please do not hesitate to contact me with any questions at the e-mail address or telephone number provided below. I look forward to sharing my conclusions with you.

Regards,
Taylor

Images of Puffy Snout

- (a) *Thunnus albacares* with a moderately-progressed puffy snout (PS) affliction.
- (b) *T. albacares* exhibiting early-stage PS.
- (c) Dorsal view of *T. thynnus* with late-stage, severe PS. Affected tissue can be seen to have progressed posteriorly.
- (d) *T. albacares* with late-stage PS. The eye can be seen to be almost completely occluded.

All images by Taylor Voorhees

APPENDIX B: LITERATURE REVIEW

Biology of Tuna

Taxonomy

Fossil records of scombrid fishes date back approximately 60 million years, to the Tertiary period. Currently, 51 extant *Scombridae* species span 15 genera. Excluding the butterfly kingfish (*Gasterochisma melampus*), four tribes exist: Scombrini (the mackerels), Scomberomorini (the Spanish mackerels or seerfishes), Sardini (the bonitos), and Thunnini (the tunas) (Graham & Dickson, 2004; Goujon & Majkowski, 2010). The 15 Thunnini species considered ‘true tunas,’ largely the focus of this study, are arranged within five genera: *Allothunnus* contains the slender tuna (*A. fallai*); *Auxis* contains the bullet (*A. rochei*) and frigate (*A. thazard*) tunas; *Euthynnus* contains the kawakawa or mackerel tuna (*E. affinis*), false albacore or little tunny (*E. alleteratus*), and black skipjack (*E. lineatus*) tunas; *Katsuwonus* contains the skipjack tuna (*K. pelamis*); and *Thunnus* contains the albacore (*T. alalunga*), yellowfin (*T. albacares*), blackfin (*T. atlanticus*), Southern bluefin (*T. maccoyii*), bigeye (*T. obesus*), Atlantic bluefin (*T. thynnus*), Pacific bluefin (*T. orientalis*), and longtail (*T. tonggol*) tunas (Collette et al., 2001; Goujon & Majkowski, 2010; Graham & Dickson, 2004). The less-formally-classified yet widely accepted group of the seven so-called ‘principal market tunas,’ (i.e., those that are most economically valuable on a global scale) are the albacore, Atlantic bluefin, bigeye, Pacific bluefin, skipjack, Southern bluefin, and yellowfin (FAO, 2011). As the scope of this study primarily extends only to those species of the *Euthynnus*, *Katsuwonus*, and *Thunnus* genera, so too are they the focus of the following sections of the *Literature Review*.

Distribution

Species of *Euthynnus*, *Katsuwonus*, and *Thunnus* genera can be found in the Atlantic, Indian, and Pacific Oceans and many of their adjacent bodies, such as the Gulf of Mexico and the Mediterranean Sea. Often, a distinction is made between tropical and temperate tunas, with an additional intermediate category for those that inhabit both climates with equal frequency and/or duration. The blackfin, false albacore, kawakawa, skipjack, and yellowfin tunas are considered tropical, as they are typically found in equatorial and sub-equatorial waters of greater than 18° C. Albacore and the three bluefin species are considered temperate; they can inhabit tropical waters (30° C), but are commonly found in waters as cold as 10° C. Bigeye tuna, considered intermediate, are found in waters 13-29° C (Collette & Nauen, 1983; Brill, 1994; Goujon & Majkowski, 2010). Most species are oceanodromous and highly migratory, but blackfin, false albacore and kawakawa tend to be more neritic (Collette & Nauen, 1983; Olson & Boggs, 1986; Goujon & Majkowski, 2010). Within each of their suitable latitudes, albacore, bigeye, skipjack, Southern bluefin, and yellowfin are circumglobal. Kawakawa inhabit the Pacific and the Indian oceans, but not the Atlantic. Atlantic bluefin, blackfin, and false albacore inhabit only the Atlantic (Collette & Nauen, 1983; Graham & Dickson, 2004). After decades of uncertainty (see Ellis, 2008), definitive evidence has revealed Pacific bluefin, as the name implies, is a distinct species and inhabits only the Pacific (Collette & Smith, 1981; Collette and Nauen, 1983; Ward et al., 1995; Smith et al., 2001).

Morphology

As precocial larvae, newly-hatched tunas are difficult or impossible to identify by morphology (Matsumoto et al., 1972; Elliot & Ward, 1995; Chow et al., 2003). A diagnostic guide, developed by Nishikawa and Rimmer (1987), can be used to distinguish scombrid species of greater than 3 mm.

Adult tunas are noted for their highly-specialised morphology (discussed here) and physiology (discussed in the following section), which aid them as strong-swimming, pelagic, predatory species. The results of such a body design are a high degree of streamlining and the capability to generate maximum thrust and lift when swimming. Generally, tunas have a fusiform body shape – somewhat elongated and slightly laterally compressed, with a relatively pointed snout. From a dorsal view, they are thickened towards the anterior with a narrow caudal peduncle, though this is most pronounced in the *Thunnus* species. All species are deepest in profile at approximately the middle of the first dorsal fin, with the exception of albacore, whose depth is greatest nearer the second dorsal fin. The two dorsal fins in all species are separated by an interspace. The first dorsal, having the ability to be completely retracted inside a slot, contains between 10 and 16 spines; kawakawa and false albacore have 10 to 15, skipjack have 14 to 16, and all *Thunnus* species have 11 to 14. The second dorsal and the anal fins each typically contain 11 to 16 rays. The first and second dorsal fins and the anal fin of all species have markedly larger anterior spines or rays than posterior, giving them strongly-concaved profiles. The second dorsal fin and anal fin in yellowfin tuna may grow notably long – to considerably greater than 20% of the fish's forked length – and are responsible for the specie's occasional

idiom, “sickle-fin.” In all species, triangular, sail-like finlets posterior to the second dorsal fin and anal fin each number 6 to 10. Pectoral fins, also retractable inside a groove, contain between 25 and 36 rays, and are typically 20 to 30% of the fish’s forked length; albacore, often called longfin albacore, have pectoral fins greater than 30% of their forked length. The paired pelvic fins are generally the smallest of all fins and, like the first dorsal, can be folded inside a slot. The caudal fin in all species is homocercal, deeply lunate in shape, and displays a species-specific aspect ratio between 6.5 and 9, among the highest of all fishes. At the anterior base of the caudal fin are a pair of large lateral keels between two smaller keels. In contrast to most other fishes, the eyes are set in the skull such that they are flush with, rather than protrude out from, the rest of the body. Tunas have very small scales or are devoid of scales almost entirely; only on the corselet and the lateral line are scales more obvious. A swim bladder is present except in kawakawa, false albacore, and skipjack, though does not form in albacore until the fish reaches 50 cm FL, and remains poorly developed thereafter (Collette & Nauen, 1983; Hebrank & Hebrank, 1986; Bushnell & Holland, 1989; Altringham & Shadwick, 2001; Westneat & Wainwright, 2001; Graham & Dickson, 2004; Goujon & Majkowski, 2010). Muscle architecture and composition are unique in tunas, and support efficient power transfer from trunk myotomes to the caudal fin (Altringham & Shadwick, 2001; Westneat & Wainwright, 2001; Graham & Dickson, 2004) and possess other, physiological capacities that distinguish them from other bony fishes.

All species display a degree of countershading, though it is most pronounced in the *Thunnus* species. False albacore and kawakawa are primarily silvery, and the

dorsal surface has several wavy, broken, oblique stripes. Ventral to the pectoral fins in these species may be dark spots; in kawakawa, 2 to 4, and in false albacore, up to 18 but typically between 3 and 7. Skipjack tuna, dark blue or blue-grey on the dorsal surface, display a series of 4 to 6 wavy stripes on the lateral and ventral surfaces. All other *Thunnus* species are shades of a metallic dark blue colour on the dorsal surface and a metallic whitish lower-lateral surface and belly. The difference in pigmentation is usually sharp. Most species have a lateral band of iridescent blue that runs along this interface, though this is faintly yellow in blackfin and bright yellow in bigeye and yellowfin. All fins are a shade of grey, blue or yellow, while the finlets are almost always yellow. (Collette & Nauen, 1983; Hebrank & Hebrank, 1986; Bushnell & Holland, 1989; Altringham & Shadwick, 2001; Westneat & Wainwright, 2001; Graham & Dickson, 2004; Goujon & Majkowski, 2010). All tuna species are dioecious, but sexual dimorphism is absent, so male-female determination is impossible by morphometrics (Schaefer, 2001).

The *Euthynnus* and *Katsuwonus* tunas are smaller in body size than the *Thunnus* tunas. Typically, these fish reach 60 to 80 cm in forked length (FL), and have not been known to exceed 100 to 110 cm. While kawakawa and false albacore are commonly 4 to 7 kg in weight, and may reach a maximum of 12 to 14 kg, skipjack are marginally heavier. Their deeper body profile allows them to commonly attain 8 to 10 kg, but individuals as large as 20 kg have been taken. Of the *Thunnus* species, blackfin are the smallest: typically 60 to 70 cm FL and 6 to 7 kg. Albacore, while only being slightly longer (50 to 110 cm FL, location-dependent), are considerably heavier; individuals of 15 to 30 kg are common and 40 kg can be reached with some

regularity. Yellowfin are the next largest, and are commonly 150 cm FL, but may exceed 200 cm FL and 175 kg. Bigeye and the three bluefin species are the largest of the tunas. Bigeye, Pacific bluefin and Southern bluefin are all commonly found at 150 to 180 cm FL and 50 to 70 kg, though their respective maximum weights appear to differ. While bigeye and Southern bluefin have not been observed greater than 200 to 260 kg, the largest of the Pacific bluefin can approach 600 kg. Atlantic bluefin, however, is the largest of all tuna species. Commonly attaining 200 cm and 350 to 550 kg, the largest individuals may reach 650 to 680 kg (Collette & Nauen, 1983; Carter et al., 2010).

Physiology

Complimenting tuna species' finely-tuned morphology are a suite of unique physiological specialisations; thermal biology, metabolic scope, and cardiac and muscle physiology each differ markedly between tunas (i.e., Thunnini) and non-tunas (Graham & Dickson, 2001, 2004; Goujon & Majkowski, 2010).

Perhaps the most well-known physiological evolution of the tunas is their capacity for endothermy. In practice, both a heat source and a mechanism for heat retention must be present. The first condition is satisfied in all fishes by the activation of slow-twitch, aerobic red muscle during swimming; in tunas, however, metabolic heat production is continuous because they never stop swimming. Heat retention, which moreover distinguishes tunas, is achieved by both the red muscle's medial positioning (i.e., internalisation), and a counter-current vascular heat exchange system, termed the *retia mirabilia*, or "miraculous network." These *retia* are bundles of intimately-positioned arterial and venous blood vessels. As venous blood leaves the

metabolically active, heat-producing red muscle, it passes by the cold, freshly-oxygenated arterial blood, and the heat diffuses across the thermal gradient (Carey et al., 1971; Stevens & Neil, 1978; Holland et al., 1992; Graham & Dickson, 2001, 2004; Korsemeyer & Dewar, 2001; Goujon & Majkowski, 2010). Graham et al. (1983) estimated that between 70 and 99% of the heat generated is conserved. Without such a system, the in-series circulation of blood in fishes and the high heat capacity of water would make heat retention impossible (Dewar et al., 1994). In addition to red muscle, this heat retention warms white muscle, viscera, the brain, and the eyes above ambient water temperature (Carey et al., 1971; Graham & Dickson, 2001; 2004). In 1983, the internal body temperature of an Atlantic bluefin tuna was measured to be 21.5° C *above* ambient (Graham et al., 1983) – the greatest observed difference to date. Amazingly, however, tunas have the ability to modify the efficiency of heat retention *in vivo*, probably by controlling the contractile state of the vessels' encapsulating smooth muscle (Graham & Dickson, 2001). Functionally, endothermy allows for the stabilisation of metabolism, digestion, acuity of sensory systems, and muscle efficiency (Bushnell & Holland, 1989; Graham & Dickson, 2004), and ecologically, it has allowed tunas to expand their vertical and latitudinal niches (Block, et al., 1993).

In relation to other fishes, including similarly-active ones (e.g., salmonids), tunas have a higher aerobic scope, more efficient oxygen transport and utilisation, a modified heart morphology and function, and unique muscle biochemistry (Graham & Dickson, 2004). First, estimations of tunas' standard metabolic rate (SMR) suggest they have as much as a ten-fold greater SMR than that of other active fishes

(Korsmeyer & Dewar, 2001), and 2 to 3 times greater than that of other scombrids (Korsmeyer & Dewar, 2001; Sepulveda et al., 2003). In addition, models (e.g., Bushnell & Brill, 1991; Brill, 1996) and experiments (e.g., Dewar & Graham, 1994) have estimated the maximum metabolic rate (MMR) of small individuals of select tuna species (skipjack and yellowfin, specifically) to be between 0.6 and 2.7 times greater than the MMR reported for several other fishes (e.g., Brett, 1972). The difference between a fish's SMR and its MMR is its aerobic scope; based on the estimations cited here, a tuna's aerobic scope (i.e., the level of aerobic activity that can be sustained before accruing an oxygen debt) is markedly higher than other active fishes. For example, Korsmeyer & Dewar (2001) use previous research to predict a 2-kg skipjack to have an aerobic scope of approximately 2000 mg O₂/kg/hr, whereas a similarly-sized sockeye salmon (*Oncorhynchus nerka*) has been measured to have an aerobic scope of 700 mg O₂/kg/hr (Brett & Glass, 1973).

The volume of water that tunas pass through their gills [3 to 6 liters/kg/min (Bushnell & Jones, 1994)] is significantly higher than that of other fish [e.g., 0.2 to 0.5 liters/kg/min for rainbow trout (*Oncorhynchus mykiss*) (Davis & Cameron, 1971; Kiceniuk & Jones, 1977)]. As such, they must be significantly more efficient at extracting the oxygen that the water contains. The gills themselves have very high surface area (7 to 9 times greater than rainbow trout) and a small diffusion distance (0.5 µm versus >6 µm in rainbow trout) (Brill & Bushnell, 2001; Olson et al., 2003; Graham & Dickson, 2004). In addition, with higher-than-average haematocrit and haemoglobin values, the blood of tunas more readily accepts diffusing oxygen than the blood of other fishes. As a result, tunas typically extract more than 50% of the water's

oxygen, where other fish species only achieve 25% to 33% efficiency (Graham & Dickson, 2004). Delivery of the freshly-oxygenated blood to the tissues is accomplished by a heart with enhanced output capabilities relative to other fishes. First, the ventricle size relative to body mass is large: 0.29% and 0.38% of body mass in yellowfin and skipjack, respectively, compared with 0.08% to 0.13% in rainbow trout and 0.11% in yellowtail (*Seriola* spp.) (Brill & Bushnell, 2001). In addition, the typical stroke volume (1 ml/kg), and ventricular and ventral aortic pressures are greater than is seen in other species (Brill & Bushnell, 2001; Graham & Dickson, 2004). Interestingly, where most fishes increase cardiac output by an increase in stroke volume, tunas use an increase in heart rate. It has been demonstrated that the routine stroke volume in tunas approaches the maximum stroke volume in other fishes, and hypothesised (Brill & Bushnell, 2001) that, because of the ventricular mass in a space-limited environment and its thick walls, it is unable to accommodate more blood in a given stroke. Thus, increasing the rate of strokes is a tuna's most efficient means of an increased output. To achieve the greater pressure, it is thought that a unique muscle fiber morphology [described by Sanchez-Quintana & Hurle (1987) and Farrell & Jones (1992)] creates an otherwise-impossible mechanical advantage.

At the muscle tissue, blood travels through elaborate capillary beds and oxygen is taken up by myoglobin. Both the density of capillaries and the content of myoglobin are greater in tunas than in non-tunas, and muscle fiber diameter is smaller, encouraging a more efficient diffusion than is otherwise typical (Korsmeyer & Dewar, 2001; Graham & Dickson, 2004). In fish, white muscle is glycogen-fuelled and anaerobic in function, creating an acidic environment after use through the production

of lactate and an oxygen debt (Korsmeyer & Dewar, 2001). Tunas, however, have more white muscle buffering capacity than other species (Perry et al., 1985; Dickson & Somero, 1987; Brill et al., 1992), allowing them to use their fast-twitch, burst-swimming white muscle for a greater duration or with more frequency than other fish. Additionally, the aforementioned high capillary density and myoglobin content allow for a higher aerobic capacity of tuna white muscle (Korsmeyer & Dewar, 2001).

Ecological Niche

Tunas are pelagic marine fish, with distributions described in detail in a previous section. The smaller tuna species (i.e., *Auxis*, *Euthynnus*, and *Katsuwonus* spp.) and juveniles of the larger ones (i.e., *Thunnus* spp.) inhabit the epipelagic zone only, typically a depth of 50 meters or less and above the thermocline (Goujon & Majkowski, 2010). While larger tunas are indeed often found at or near the surface, they may also frequent waters of the mesopelagic zone; during feeding dives, yellowfin (Dagorn et al., 2006), bigeye (Goujon & Majkowski, 2010) and Atlantic bluefin (Block et al., 2005) all may exceed depths of 1,000 meters. It is their physiological specialisations, most notably the capacity for endothermy, which make these feats possible. Most tunas school according to size; juveniles of large tuna species may school with individuals of smaller tuna species, but adults of larger species school with similar-sized conspecifics. Schools may be a few tens of fish to a few thousand fish in number. Tunas school in search of food, for seasonal migrations, and, by the smallest among them, for protection from predators (Goujon & Majkowski, 2010). As obligate ram ventilators, a cessation of swimming activity would result in suffocation (Brown & Muir, 1970; Korsmeyer & Dewar, 2001; Goujon

& Majkowski, 2010). Furthermore, their inherent negative buoyancy requires them to swim at a minimum speed in order to provide enough lift to maintain hydrostatic equilibrium (Magnuson, 1978; Magnuson & Weininger, 1978). Based on studies which recorded routine swimming speed in tunas in the laboratory or in the field (e.g., Dizon et al., 1978; Dizon & Brill, 1979; Block et al., 1997; Brill et al., 1999; Freund, 1999), it has been estimated that their minimum swimming requirement is a rate of 1 to 2 bodylengths/second (Brill & Bushnell, 2001).

Many of the tuna species undergo seasonal migrations to capitalise on optimal food resources or spawning conditions. The larger, temperate tunas (i.e., albacore and the three bluefin species) typically move thousands of kilometers from prime foraging grounds in cooler waters to tropical waters for spawning, whereas the tropical (i.e., yellowfin) and more neritic (i.e., blackfin, false albacore, kawakawa) tunas have more limited long-distance movements (Goujon & Majkowski, 2010). A 1988 review of tagging studies (Joseph et al., 1988) reported Atlantic bluefin, albacore, skipjack, and Pacific bluefin to have each been recaptured 7,700 km, 8,500 km, 9,500 km, and 10,790 km, respectively, from the locations of tag implantation.

Adult tunas are opportunistic feeders and amongst the top predators in their ecosystems. All tuna species prey on other pelagic and epipelagic fish, cephalopods, and crustaceans (Olson & Boggs, 1986; Roger, 1994a,b; Goujon & Majkowski, 2010), though larger tunas often dive into mesopelagic waters for foraging (Holland et al., 1992; Ménard et al., 2000; Allain, 2005). Analyses of stomach contents have led to the notion that tunas are non-selective feeders; dozens of species have been identified (e.g., *Scomber* spp., *Auxis* spp., *Vinciguerria* sp., *Cubiceps* sp., *Stolephorus* sp.,

Myctophum spp., *Loligo* spp., *Ilex* spp., Gonostomeidae spp., Euphausiid spp., Amphipod spp., Tunicada spp.) as having been preyed upon by tunas (Perrin et al., 1973; Olson & Boggs, 1986; Roger, 1994a,b; Ménard et al., 2000; Bertrand et al., 2002; Chase, 2002; Goujon & Majkowski, 2010). The size of feed items is generally positively correlated with a tuna's body size. Larvae and post-larvae feed on zooplankton and other fish larvae (Goujon & Majkowski, 2010). At least in juveniles and adults, feeding may occur at any hour of the day or night (Bard et al., 1998; Goujon & Majkowski, 2010; T. Voorhees, 2012, 2013, unpublished data). With respect to food web positioning, larval and post-larval tunas are preyed upon by zooplankton foragers and early juveniles of other fish, smaller tunas and juveniles of larger species are preyed upon by larger pelagic predatory fish, sharks, dolphins, and toothed whales, and large adult tunas are preyed upon almost exclusively by large pelagic sharks and toothed whales (though, humans play an undeniably integral role in 'preying' on large tunas) (Goujon & Majkowski, 2010).

Like most marine finfish, tunas are oviparous, have asynchronous oocyte development, and are batch spawners. As broadcast spawners in near-surface waters, they rely on external fertilisation of the females' eggs by the males' sperm. As mentioned in the Morphology section, they are dioecious and exhibit no sexual dimorphism (Schaefer, 2001). Tropical tunas typically spawn year-round in equatorial waters and during the warmest few months of the year in higher latitudes. Temperate tunas, however, have distinct spawning seasons, and often undergo long-range migrations from foraging grounds to spawning areas. The three bluefin species are especially recognised for this phenomenon. Southern bluefin move, during

September and October, from cool waters off Tasmania and New Zealand to their warmwater spawning grounds off west- and northwest Australia, where they will remain until March. Pacific bluefin, inhabiting all waters from Baja California to California to the Okhotsk Sea to the Sea of Japan, migrate to waters northeast of the Philippines and the South China Sea for spawning (Collette & Nauen, 1983; Bayliff, 1994; Inagake, 2001). There is also evidence of a PBT spawning ground in the Sea of Japan (Bayliff, 1994; Inagake, 2001; Nakadate et al., 2011). The greatest attention, however, has been focused on the migration and spawning habits of Atlantic bluefin. It is now known that while ABT are, for much of the year, homogenously distributed throughout the Atlantic Ocean (Block et al., 2001; Block et al., 2005; Rooker et al., 2008), two distinct spawning grounds each host a distinct ABT stock every boreal summer (Mather et al., 1995; Block et al., 2001; Block et al., 2005). In the Mediterranean Sea, spawning occurs between May and August, whereas in the Gulf and Mexico (and potentially the Bahamas), spawning occurs between April and July (Dicenta & Piccinetti, 1980; Cort & Loirzou 1990; Richards, 1990). It has been hypothesised that at least one alternate ABT spawning ground exists (Lutcavage et al., 1999; Goldstein et al., 2007; Galuardi et al., 2010), but no definitive evidence has confirmed this.

Skipjack, false albacore, and kawakawa tuna become sexually mature when they reach 40 to 50 cm FL, usually at two years of age (Collette & Nauen, 1983; Goujon & Majkowski, 2010). Blackfin tuna may become mature at 40 to 50 cm FL, but all will reach maturity before attaining 60 cm FL (Collette & Nauen, 1983; Vieira et al., 2005; Gardieff, 2014). Albacore reach maturity at five years of age, 90 cm FL, and 15 kg in

weight. Yellowfin and bigeye tuna both reach maturity at 100 to 110 cm FL; in yellowfin, this is typically at 2.5 to 3 years of age and 20 to 30 kg in weight (Collette & Nauen, 1983; Goujon & Majkowski, 2010). Pacific bluefin are thought to mature between 3 and 5 years of age (Bayliff, 1994; Chen et al., 2006b; Tanaka et al., 2006), but fish caught in spawning grounds predominately measure greater than 160 cm FL, suggesting this parameter be considered the most reliable (Sawada et al., 2005; Itoh, 2006; Shimose et al, 2009). The two stocks of Atlantic bluefin are believed to reach maturity at different ages and sizes. There is evidence that fish of the eastern ABT stock (i.e., those spawning in the Mediterranean Sea) mature at 3 to 5 years of age, 115 cm FL, and 30 kg in weight (Collette & Nauen, 1983; Goujon & Majkowski, 2010; Boustany, 2011), whereas those fish of the western stock (i.e., those spawning in the Gulf of Mexico) mature much later. It has been suggested that western stock fish no younger than five years of age, and most likely at least eight years of age, 190 cm FL, and 120 kg in weight are sexually mature (Baglin, 1982; Mather et al., 1995; Block et al., 2005; Heinisch et al., 2008; Boustany, 2011). As is typical of marine finfish, the per-batch fecundities of female tunas are in the several-hundred-thousands to millions (Schaefer, 2001; Goujon & Majkowski, 2010; de la Gándara et al., 2011; Bezerra et al., 2013).

The longevity of tunas is species-specific but, as is typically seen elsewhere in biology, the larger species tend to live longer than the smaller species. False albacore, blackfin, and kawakawa each typically live between 3 and 7 years, but potentially as long as 10 years (Collette & Nauen, 1983; Goujon & Majkowski, 2010; Bester, 2014; Gardieff, 2014). Skipjack are estimated to live 8 to 12 years. Yellowfin and albacore

probably live 12 to 15 years (Collette & Nauen, 1983). The three bluefin species are the longest-lived of the tunas; all attain 20 years of age with regularity (Collette et al., 2011). Pacific bluefin may live up to age 26 (Boustany, 2011). The longevity of Atlantic bluefin may be different for the eastern and western stocks, but estimates of maximum age for western-stock ABT have been as old as 32 years (Mather et al., 1995; Neilson & Campagna, 2008; Boustany, 2011). Southern bluefin, thought to have the longest potential life cycle, may reach 40 years of age (Farley et al., 2007; Gunn et al., 2008; CCSBT, 2010; Boustany, 2011).

Tuna Fishing, Market, Current Stock Status, and Management

Tuna Fishing and Fisheries

The first tuna fisheries, dating back thousands of years, involved artisanal fishing along the coasts where tunas are found. Fish were captured using traps, nets and hand-lines, and sold fresh, smoked and salted to local markets (Sara, 1980; Fromentin, 2009; Miyake, 2005; Miyake et al., 2010). As the demand for tuna increased in the 1940s, '50s, and '60s, particularly of the canned variety, a second phase in tuna fisheries emerged. While artisanal fishing continued much the same, commercial fleets became markedly more industrialised, traveling farther offshore and employing purse seines, longlines and baitboat fishing methods. Geographic expansion was rapid, as European fleets fished the tropical Atlantic off West Africa and Japanese fleets could be found in every ocean throughout the world. Catch data began in earnest in 1950. In the early 1950s, the global total capture of tuna and tuna-like species was less than 0.6 million tonnes, approximately three-quarters of which were

the principal market tunas (i.e., skipjack, yellowfin, bigeye, albacore, and the three bluefin species) (Miyake et al., 2010; FAO, 2011, 2013). The Pacific Ocean provided the majority percentage of the catch, and the Japanese fleet was responsible for more than half of all tunas captured (Miyake et al., 2010; FAO, 2011).

Since 1950, the annual global capture of tuna species has steadily increased. Skipjack and yellowfin have historically been the most- and second-most-captured species, and catches of each have continually trended upward, though yellowfin catches appear to have peaked in 2003 and fallen slightly since then. Of the rest of the principal market tunas, albacore began the 1950s as the most-captured and catches have increased since, but landings of bigeye have overwhelmingly shown the most growth. Bigeye catches surpassed albacore catches in the mid-1970s and modifications to longline technology have resulted in substantial increases since the mid-1980s. Like yellowfin, however, bigeye catches appear to have peaked and subsequently declined over the past decade. The three bluefin species have all experienced variable catch numbers, though always much less than the other principal market tunas. Catches of Atlantic bluefin declined from the 1950s through the 1970s, remained stagnant, increased steeply in the early 1990s, and have declined again since then. The highest catch of Pacific bluefin was achieved in 1956, the lowest in 1990, and has been historically sporadic. Southern bluefin catches rapidly increased in the 1950s, varied in the '60s and '70s, fell sharply in the '80s, and have thus far remained without trend. The Pacific Ocean continued to be the highest-producing of the three, and catches in the Indian Ocean surpassed those in the Atlantic in the mid-1980s. With respect to gear type, the advent of purse seining has been the most notable

change in tuna fishing. Though growing steadily from the 1950s to the mid-1970s, it thereafter exploded and became overwhelmingly the highest-landing gear type.

Though initially the leading gear type, baitboats have shown a declining trend in terms of proportional contribution. Though significantly important through the late 1970s, longline contributions have also declined on a percentage basis. Trolling, though never significantly productive, also became nearly obsolete on a global scale in the 1980s and has remained so since (Miyake et al., 2010; FAO, 2011).

Currently, more than four million tonnes (4.4 million tonnes in 2010) of principal market tunas are captured annually (Miyake et al., 2010; FAO, 2011, 2013). Skipjack accounts for the greatest proportion, (50.7 percent of global total, 2.5 million tonnes in 2009), followed by yellowfin (31.7 percent, 1 million tonnes) and bigeye (10.8 percent, 0.4 million tonnes) (Miyake et al., 2010; FAO, 2011). The three bluefin species continue to contribute relatively little in terms of volume (a combined 50,000 tonnes in 2010), each approximately 1 percent of global total (FAO, 2014), though the price they fetch renders them one of the most valuable. The Pacific remains the largest producer of tunas, contributing almost two-thirds of the global total, while the Indian and the Atlantic contribute one-quarter and 10 percent, respectively (Miyake et al., 2010; FAO, 2011). Purse seine fisheries capture between 40 and 70 percent of the global total, with proportionately more in the Pacific, and longline and baitboat operations each contribute between 8 and 25 percent annually (Miyake et al., 2010).

It should also be recognised that non-commercial fisheries for tuna species are active and important. In the United States, Mexico, Australia, South Africa, and several Central American nations, private recreational and professional charter tuna

fishing have become immensely popular, developing an industry in the process; the extent of which warrants catch-volume-monitoring and regulation by management authorities. In the case of bluefin, for example, recreational landings of Pacific bluefin between 1987 and 2011 were highly variable, but had a mean of 200 tonnes annually (ISC, 2014), and in the Atlantic, 182 of the 957 tonnes of Atlantic bluefin quota for 2011 were allocated to the recreational sector (NOAA, 2011). Of the remainder of the principal market Atlantic tunas, only yellowfin carry a daily bag limit (three fish per person per day), and both yellowfin and bigeye have a minimum at which they can be kept (27 inches curved forked length) (NOAA, 2014). And while contributions in terms of volume may be relatively little, local and regional economic impacts are significant. In the Atlantic, for example, it was estimated that private tuna-fishing activities between Maine and North Carolina alone had an economic impact of nearly US\$9 million in 2011 (NOAA, 2014). A 1997 study of the recreational Atlantic bluefin fishery near Hatteras, North Carolina, USA revealed a local impact of more than US\$4.5 million (Bohnsack et al., 2002). Economic value for the same sector in eastern Australia has been estimated at almost AUS\$7.5 million annually (Galeano et al., 2004).

Market for Commercial Tuna Fisheries

The driving factor for any commercial fishing industry is the market, and the tuna industry is a premium example. The rising demand for canned tuna, beginning in the 1940s and '50s, and for fresh tuna, in the 1990s and 2000s, provided two significant increases in market value. Currently, tunas caught for canning (predominantly albacore, yellowfin and skipjack) fetch between US\$1.60 and US\$3 per kilogram at

landing. This relatively low per-kilogram price is offset by a large volume. By contrast, tunas captured for the fresh market (predominantly bluefin and, increasingly, bigeye, with some yellowfin) are lower in volume but command a high per-kilogram price. Of this non-canned sector, there are two submarkets: the sashimi market, to which the highest-valued fish go, and the non-sashimi fresh and frozen market (Miyake et al., 2010; FAO, 2011). A fish's 'grade' (i.e., its value), and thus the market it supplies, is dependent on the fat content, colour, and texture of its muscle (Bartram et al., 1966; McConnell & Strand, 2000; Chubby Fish, 2014). Fish supplied to the sashimi market routinely fetch US\$25 to US\$40 per kilogram, with some approaching US\$100 per kilogram. At certain times of the year, fish of exceptional quality may fetch US\$500 per kilogram or more (FAO, 2011). In 2010, the value-at-landing of the principal market tunas was more than US\$10 billion (FAO, 2013).

Tuna Species Stock Status

The current status of wild tuna populations and the future implications of current fishing pressures relative to those population statuses was thoroughly explored by Miyake et al. (2010), and included in the FAO's most recent *Review of the state of world marine fishery resources* report (2011). Of the principal market tunas, most are considered to be "fully exploited," some "overexploited," and some "not fully exploited"; it has been concluded that different geographical stocks of the same species may be of different exploitation statuses (FAO, 2011). In general, the temperate species are more at risk for overexploitation than their tropical cousins (Miyake et al., 2010; FAO, 2011).

The only principal market tuna to be considered not fully exploited in all oceans is skipjack, though the east Pacific stock is considered to be fully exploited. Both bigeye and yellowfin are considered to be fully exploited in all oceans, but yellowfin in the west Pacific are considered to be not fully exploited. The status of albacore is the most variable, and considered to be not fully exploited in the south Pacific, fully exploited in the Indian and the south Atlantic, and overexploited in both the north Pacific and the north Atlantic. Southern bluefin is overexploited in all oceans, and the Atlantic and Pacific bluefins are also considered to be overexploited in their respective oceans (FAO, 2011).

Miyake et al. (2010) took stock status analysis a step further, and quantified the degree to which each species was under- or over-exploited. They reasoned that a specie's stock status can be evaluated, in the simplest manner, by calculating the ratio of its current spawning stock biomass (SSB) to the necessary SSB thought to support and maintain the maximum sustainable yield (MSY) (i.e., $SSB_{current} : SSB_{MSY}$). A ratio less than 1.0 would indicate the given specie's population is lower than is thought to be sustainable, whereas a ratio higher than 1.0 would indicate there are more than enough spawning individuals to maintain the specie at that reference point. While the FAO (2011) report uses terms rooted by "exploitation," Miyake et al. (2010) opted for the synonym "overfishing." Concurring with the FAO (2011) report, skipjack were considered not to be overfished, with estimated stock statuses over 1. Bigeye was considered less plentiful in the Atlantic (0.92) than in the Indian or Pacific (1.01 to 1.37, depending on stock), and estimates of all stocks of yellowfin are near to 1. Estimates of the status of albacore were also generally in accordance with FAO

estimations, though minor differences can be seen. Stocks in the north and south Atlantic are estimated to be low (0.81 and 0.91, respectively), where stocks in the Indian and the north and south Pacific are all considered to be adequate (>1). With regard to bluefin, the degree of poor stock health is clear, as $SSB_{current} : SSB_{MSY}$ of Atlantic bluefin is estimated to be between 0.14 and 0.57, and of Southern bluefin, 0.101 to 0.127. Interestingly, their estimates for Pacific bluefin stock health are less clear, as they contest that a reference point is not defined, though they do recommend that the rate of fishing mortality should not be increased (Miyake et al., 2010).

Tuna Fisheries Management

Management of tunas has proven difficult and, at times, controversial. Four reasons contributing to this difficulty are: a) tunas are highly migratory and regularly cross boundary lines which limit regulatory agencies' jurisdictions, b) industrial fishing fleets that target tuna species are highly mobile and can fish in areas previously inaccessible or historically foreign, c) tunas are highly valuable and regularly traded on a global scale, and d) the marked increases in demand and value have potentially encouraged Illegal, Unreported, and Unregulated (IUU) fishing. For these reasons, tunas (and many of their pelagic cohorts) were given special consideration during the 1982 United Nations Convention on the Law of the Sea (UNCLOS). They were categorised as “highly migratory species,” and it was determined that their management should be a collaborative effort between states with regional councils as the functional unit of collaboration (Allen, 2010; Miyake et al., 2010; FAO, 2011). Five regional fisheries management organisation (RFMOs) bodies exist to manage their respective region's tuna fisheries; the International Commission for the

Conservation of Atlantic Tunas (ICCAT) has jurisdiction in the Atlantic Ocean and its adjacent seas, the Inter-American Tropical Tuna Commission (IATTC) has jurisdiction in the Pacific Ocean east of longitude 150° W, the Western and Central Pacific Fisheries Commission (WCPFC) has jurisdiction in the Pacific Ocean west of longitude 150° W, the Indian Ocean Tuna Commission (IOTC) has jurisdiction in the Indian Ocean, and the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) has jurisdiction in the South Atlantic Ocean from Argentina to Namibia, in the Indian Ocean from Moçambique to northern Australia, and in the South Pacific Ocean in the waters southeast of Australia and surrounding New Zealand. The CCSBT's reach overlaps with that of ICCAT, WCPFC, and IOTC. These five RFMOs exercise collaboration with each other under the auspices of the UN's FAO (Allen, 2010; Miyake et al., 2010; FAO, 2011). It should be noted, however, that although the 1982 UNCLOS meeting set collaboration amongst the bodies into motion, many of the bodies themselves existed beforehand. For example, as the first tuna body, the IATTC was formed in 1950 (Allen, 2010). Recent, thorough examinations of tuna fisheries management were done by Allen (2010) and Miyake et al. (2010).

Commercial Farming of Tunas

Capture-based commercial tuna aquaculture ventures exist in Japan, Mexico, Australia, and throughout the Mediterranean Sea (Farwell, 2001; Carter et al., 2010; Partridge, 2013). These 'tuna ranching' endeavours, targeting primarily bluefin tuna (*Thunnus thynnus*, *Thunnus orientalis*, and *Thunnus maccoyii*), use purse seines to

capture entire schools of wild fish, and tow the catch to nearshore sites where they are transferred into sea-surface cages. At a tow speed of approximately one knot, the trip from fishing grounds to nearshore operating site can last several weeks. The fish are held in the cages for several months and fattened on baitfish (i.e., sardine, pilchard, herring, mackerel) before being harvested and sold (Miyake et al., 2003; De Stefano and Van Der Heijden, 2007; Ottolenghi, 2008; Mylonas et al., 2010). The cages are typically circular and 30-90 meters in diameter, 10-20 meters deep (Lioka et al., 2000; Farwell, 2001; Ottolenghi, 2008; Carter et al., 2010). Daily feeding rates vary between 2% and 10% of the fish biomass, and this ration is allocated over one to three feedings per day (Farwell, 2001; Ottolenghi, 2008). Harvests are typically conducted by isolating a select number of fish from the rest of the population, and utilising trained divers to shoot the fish with a power-head (*lupara*) from underwater, trained marksmen to shoot the fish with a single-bullet shotgun or *lupara* from the surface, or by lethal concussion (Mylonas et al., 2010). The process of on-growing wild-caught tunas increases their per-kg value by relatively rapid weight gain and an increase in fat content (highly desirable by consumers and thus, largely responsible for a tuna's 'grade'), by controlling the supply to market, and, to a lesser extent, by managing risk associated with fluctuating rates of currency exchange. Japan is the primary market for bluefin tuna, though markets in China, Europe and the United States have expanded considerably in recent years. The fish is consumed almost exclusively as sushi and sashimi (Carter et al., 2010).

Atlantic Bluefin Tuna (*Thunnus thynnus*)

Atlantic bluefin tuna (ABT) were the first of the tuna species to be on-grown in captivity after capture for commercial purposes. After migrating northwards in the spring and early summer from their spawning grounds in the Gulf of Mexico, ABT are often lean and of little commercial value by the time they reach Canada. In the late 1970s, Nova Scotian fishermen decided to enhance the value of these early-season fish by herding them from the mackerel traps in which they were caught into large holding pounds. Here, the fishermen fed the fish throughout the summer to improve their weight and condition. Additionally, creating ready-access to the fish would allow the fishermen to take advantage of a fluctuating market. When the price was right and the fish were fat, the harvest would be sold fresh to the Japanese market (Buchanan, 1977; Carey et al., 1984, Farwell, 2001; Carter et al., 2010).

Currently, ABT ranching occurs throughout the Mediterranean Sea. Beginning in the mid-1990s, production has grown to more than 60,000 tonnes (t) annually, with 60 total facilities and Spain, Malta, Turkey, Italy, and Croatia being the chief producers (Ottolenghi, 2008; Carter et al., 2010; ICCAT, 2014.). The ICCAT, in addition to their oversight of fishing activities of Atlantic tunas, is responsible for the management of ABT ranching operations. The ICCAT makes a distinction between “fattening” and “farming” of tunas. Fattening operations capture sexually mature fish during their June and July post-spawning migration out of the Mediterranean Sea. These two- to four-year-old fish, typically weighing between 40 and 200 kg (but at least, as regulations require, 30 kg and 130 cm FL), are captured primarily in purse seines and typically on-grown for three months to two years (Farwell, 2001; Ticina et

al., 2004; Carter et al., 2010; Mylonas et al., 2010). In contrast, ABT farming operations, located only in Croatia due to a ‘grandfather clause’-type agreement and their relatively small contribution to total ABT ranching production, capture juvenile fish for on-growing. These sexually immature fish, with a weight of 8 to 20 kg at capture, are held for two to three years until they reach their harvest weight of 30 to 50 kg (Ticina et al., 2004; Ticina et al., 2007; Mylonas et al., 2010).

Southern Bluefin Tuna (*Thunnus maccoyii*)

Though Atlantic bluefin tuna were the first of the tunas to be on-grown, the Southern bluefin (SBT) was the subject of the industry’s rapid expansion. In response to declining stocks and tightening quotas, SBT ranching began in Port Lincoln, South Australia in 1991 by capturing fish in the Great Australian Bight (Farwell, 2001; Nowak et al, 2006; Carter et al., 2010; Kirchhoff, 2012). During the two initial years of the effort, fish were captured by pole fishing and transported to the farm site in the boats’ in-deck bait-holding tanks. Small transport capacity and high mortality, however, encouraged innovation, and the currently-employed method of purse seine capture and transport was developed (Nowak et al., 2006). Currently, large schools of two- to four-year old, 17 to 20 kg juvenile fish are captured between December and March of each year and, after a 10- to 20-day tow, transferred to their holding pens in the Tuna Offshore Farming Zone near Port Lincoln (Farwell, 2001; Nowak et al., 2006; Kirchhoff, 2012). After three to eight months of being fed baitfish and a commercially-available “wet pellet” (Glencross et al., 1999), fish are harvested at up to twice their weight at stocking (Carter et al., 2010). Nearly all of Australia’s SBT catch quota (5,151 t of the 12,449 t global total for 2014) is reserved for ranching

operations (Kirchhoff, 2012; CCSBT 2014a), and is valued between AU\$180 million and AU\$300 million (~USD\$165-275 million) annually (Gardner et al., 2006; Kirchhoff, 2012; CCSBT, 2013). As of March 2014, there are 16 licensed SBT ranching operations in Australia, most of which maintain multiple sites (CCSBT, 2014b).

Pacific Bluefin Tuna (*Thunnus orientalis*)

On-growing of Pacific bluefin tuna (PBT) began in Japan the 1970s, shortly after the Canadian efforts (Kumai, 1997; Masuma et al., 2008). Presently, PBT are on-grown in both Japan and Mexico. In Japan, young-of-the-year juveniles, between 220 and 400 g in weight, are captured on the troll with barbless hooks (Farwell, 2001; Masuma et al., 2008; Carter et al., 2010) and, more recently, by purse seine (Masuma et al., 2008). Over three to four years the fish are fed locally-source baitfish, and are harvested upon reaching an approximate weight of 40 kg (Farwell, 2001; De Stefano & Van Der Heijden, 2007; Carter et al., 2010). In contrast to the operations in Australia and the Mediterranean Sea, where survival from capture to harvest is generally 90% or better, Japanese PBT on-growers often experience only 30% overall survival (Masuma et al., 2008). In Mexican and Californian waters, two- to four-year old fish, weighing between 20 and 50 kg each, are captured from June to September (Farwell, 2001; Smart & Sylvia, 2006; Carter et al., 2010). They are grown in the coastal waters of Baja California, Mexico for three to nine months before harvest. Smart and Sylvia (2006) reported, in their assessment of North American bluefin tuna culture, that eight Mexican farms produced 3,800 tonnes during the 2004-2005 season, and Ukawa and Takii (2006) reported Japanese farms to have had recently harvested

approximately 2,400 tonnes in a year. The global Pacific bluefin ranching industry seems to have undergone significant growth, however; according to the FAO time-series of PBT aquaculture production, after never having exceeded 3,500 tonnes of global production in a single year, nearly 11,500 tonnes were produced in 2012 (FAO, 2014).

Areas of Improvement and Future Direction of Tuna Aquaculture

One area that has garnered much attention from ranching industry researchers is feed. Currently, captured baitfish make up the large majority of feed items given to on-grown tunas. For a number of reasons, efforts to replace some of this ration with commercially-produced artificial feeds are ongoing. First, as a result of the industry's expansion, the cost of these baitfish has increased markedly; for example, in just five years, from 1998 to 2002, sardine prices doubled (De Monbrison & Guillaumie, 2003; Volpe, 2005). Second, there is concern regarding the stability and resiliency of these baitfish stocks under increased fishing pressure (Anonymous, 2005; Tacon and Metian, 2009). Third, as the nutritional quality of the baitfish varies seasonally, annually, and with respect to location, inconsistencies in nutrient uptake by the tunas is of concern (Van Barneveld and Vandeppeer, 2007; Ottolenghi, 2008). Fourth, if baitfish are fed without prior freezing, as is often the case in Japan, there is the potential for disease transmission to the tunas being on-grown. Finally, and which magnifies these issues, because tunas have such high metabolic demands, [Korsmeyer & Dewars (2001) estimate as little as 5% of energy intake is used for growth], the volume of feed required to attain the desired growth and body-fat percentage is more than considerable (Ottolenghi, 2008). In 2006, for example, it was estimated that as

much as 300,000 tonnes of these forage fish were consumed by tuna ranching operations globally (Huntingdon, 2008; Tacon and Metian, 2009), while producing less than 15,000 tonnes of tuna (FAO, 2008)

This wild-capture, on-growing method of tuna farming, while maximising yield in the immediate term, ultimately requires the continued collection of fish from the ecosystem. Furthermore, the ecological effects of this removal may be exacerbated by the fact that a large proportion of those tuna captured are of pre-reproductive size (Aranda et al., 2011; Farwell, 2001; Masuma et al., 2008; Miyashita et al, 2000; Mylonas et al., 2010; Ottolenghi, 2008). As such, much effort has recently been dedicated to the artificial propagation (i.e., ‘closed-loop’ or ‘closed-cycle’ aquaculture) of tunas – a strategy that has been successful for numerous other species (e.g., Atlantic salmon (*Salmo salar*), barramundi (*Lates calcarifer*), European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), yellowtail kingfish (*Seriola lalandi*)) in consistently supplying the market with minimal dependence on wild stocks – and is deemed the future of tuna aquaculture. Sawada et al. (2005) cited the variation in quality of wild-caught juveniles and reductions in fishery quota to be the chief incentives of this movement. To attain this, captive broodstock must produce larvae, which, in turn, are grown out to a harvestable size. While larval rearing has been identified as a major bottleneck in the development of tuna aquaculture, so too has the achievement of maintaining a consistently-spawning, captive population of broodstock (see 2nd Global COE Program Symposium, 2009; Joint International Symposium, 2010). Furthermore, advancements in larval rearing technology and methodology would be best achieved with a consistent supply of high-quality fertilised

eggs. Looking forward, commercial aquaculture operations rely on such consistency to supply the market.

Research and Land-Based Holding of Tunas

Tuna species have been kept in land-based captivity for several decades, for research, commercial, and public display purposes. Their popularity as a food-fish has rendered them among the highest-valued commodities on Earth, their unique physiological capabilities have been of interest to scientists worldwide, and their graceful majesty makes them an asset to any aquarium.

The first attempts at land-based holding of tuna species were carried out as early as 1951 at the Kewalo Basin Research Facility on the south shore of Oahu, Hawai'i, USA (Nakamura, 1972; Brill, 1999, 2002; Farwell, 2001). With the intention of studying life history and physiology, this group routinely kept juvenile (1-4 kg) yellowfin (*Thunnus orientalis*), skipjack (*Katsuwonus pelamis*) and kawakawa (*Euthynnus affinis*) tuna in shoreside tanks (Dizon and Sharp, 1978; Farwell, 2001; Brill, 1999, 2002). These efforts were successful in determining many of the husbandry requirements of tunas, and undoubtedly paved the way for future work necessitating live-holding.

In the early 1970s, researchers at the Fisheries Institute of Kinki University and at the Marine Science Museum of Tokai University, both in Japan, were the first to hold tuna species in captivity for aquaculture research (breeding and larval rearing), specifically (i.e., research with a commercial purpose) (Farwell, 2001; Harada, 1973; Harada et al., 1971a,b, 1973a,b, 1980; Suzuki et al., 1972). In 1979, captive bluefin

tuna broodstock spawned for the first time in history (Kumai, 1997). Since then, several groups within Japan have had success in egg collection and fingerling production. A unified body of collaborating institutions, the Japan Sea Farming Association (now Fisheries Research Agency), was initiated in 1991, and cooperation and information exchange has been beneficial. In 2002, Sawada et al. (2005) were the first to successfully close the Pacific bluefin tuna (*Thunnus orientalis*) life cycle. In this, artificially-hatched (F1 generation) fish spawned a second generation (F2) of healthy larvae that were reared to the juvenile stage. Currently, research sites of Kinki University are harvesting artificially-hatched, captively-reared fish each year (Masuma et al., 2008).

Clean Seas Tuna, Ltd., in Port Lincoln, South Australia, initiated a Southern bluefin tuna (*Thunnus maccoyii*) lifecycle closure program in 2005. Since then, they have had mild success in obtaining fertilised eggs and have conducted several larval rearing trials. In 2010, they reported to have produced 100,000 Southern bluefin tuna fingerlings, the oldest of which survived more than 200 days-post-hatch (Clean Seas, 2010). In December, 2012, however, the project was suspended due to sub-par production and an attempt to rehabilitate its other endeavours, kingfish production and bluefin tuna ranching (Clean Seas, 2012).

Since 1985, the Achotines Laboratory in Los Santos Province, Republic of Panama, has been investigating the early life history of tuna species. In 1993, a concerted effort began with the intention of maintaining yellowfin tuna broodstock in land-based tanks. They succeeded in establishing a spawning population (Wexler et al., 2003), and near-daily spawning allows for the continuation of this research to date.

Specific investigations include husbandry and spawning protocols (e.g., Wexler et al., 2003; Margulies et al., 2007), and basic early life history characteristics such as growth, temperature and dissolved oxygen requirements, diet, and the ontogeny of visual capabilities and the digestive system (e.g., Margulies et al., 2001; Wexler et al., 2001; Margulies et al., 2007; Wexler et al., 2007; Buentello et al., 2011; Wexler et al., 2011; Margulies et al., 2013). In Bali, Indonesia, the Australian Centre for International Agriculture Research sponsored an investigation into the development of a consistently-spawning yellowfin tuna broodstock population at the Gondol Research Institute for Mariculture, which has been the site of yellowfin tuna aquaculture research since 2003. The first incidence of spawning was in 2004, and eggs have been collected several times since then, though low numbers of eggs, low fertilisation rates, and pathogenic infections have hindered progress of the development of larval rearing protocols (Hutchinson et al., 2012). At the University of Miami's Rosenstiel School of Marine and Atmospheric Science, efforts have been underway to establish a blackfin tuna (*Thunnus atlanticus*) broodstock population (Benetti et al., 2009), though no success in lifecycle closure has been reported.

Since 1994, the Tuna Research and Conservation Center (TRCC) in Pacific Grove, California, USA, has held several tuna species in land-based captivity for husbandry and physiology research. A collaboration between Stanford University's Hopkins Marine Lab and the Monterey Bay Aquarium, the TRCC routinely holds more than 100 tunas (yellowfin, skipjack and Pacific bluefin) at a given time (Farwell, 2001, 2003).

Most recently, the University of Rhode Island has entered a research agreement with GreenFins, LLC, a Rhode Island-based company with the goal of developing the aforementioned ‘closed-loop’ aquaculture of yellowfin tuna. Wild fish were successfully captured and transported to the land-based broodstock facility, but as of October, 2014, no eggs have been collected (Voorhees, 2014, unpublished).

Public Display

In addition to the efforts to establish land-based holding for research purposes and commercial production, there has been effort to hold tuna species in aquariums for their display to the public. As of 2001, there were seven such operations: six in Japan (Tokyo Sea Life Park, Nagoya Port Aquarium, Kaiyukan Aquarium, Aburatsubo Marine Park, Kagoshima, and Aqua-Marine Fukushima) and one in the United States (Monterey Bay Aquarium) (Farwell, 2001). These efforts afford the general public a rare opportunity to witness first-hand the majesty and uniqueness of tuna, and create a tangible association with an otherwise-inaccessible animal.

The Stress Response in Teleost Fishes

In response to a stressor, fish exhibit a suite of physiological changes and behavioural modifications in an attempt to cope with the demand imposed on it. The stress response itself is manifested at multiple levels of organisation (from molecular responses to community structure) and as such, is commonly categorised into the primary, secondary, and tertiary responses (Barton and Iwama, 1991; Barton, 1997, 2002; Iwama, 1998).

The process of stress coping begins with the central nervous system's recognition of a real or perceived threat to homeostasis (Barton, 2002). Upon such recognition, the stimulation of chromaffin cells, located in the anterior kidney of teleosts, to release catecholamines – primarily epinephrine – is achieved through those cells' direct innervation (Reid et al., 1996, 1998, Barton, 2002). This innervation, along with catecholamine storage within the chromaffin cells, allows for a rapid release, and circulating levels increase instantly with stress (Mazeaud et al., 1977; Barton, 2002). Delayed by several minutes relative to catecholamine release, but nonetheless part of the primary response, is the release of cortisol by way of the hypothalamus-pituitary-interrenal (HPI) axis (Barton and Iwama, 1991; Iwama, 1998; Barton, 2002). Corticotropin-releasing hormone (CRH), originating in the brain's hypothalamus, stimulates the release of adrenocorticotropin (ACTH) from the anterior pituitary which, in turn, acts on interrenal cells of the anterior kidney to release cortisol, among other corticosteroids (Iwama, 1998; Barton, 2002; Iwama et al., 2005).

The secondary response to stress is characterised by changes in numerous homeostatic regulatory processes. As stress is an energy-demanding process, energy stores must be mobilised to meet that demand (Barton, 1997, 2002). Circulating levels of glucose are increased through glycogenolysis and/or gluconeogenesis in the liver so that they may be used by tissues integral to the 'escape' or coping of the stressor, such as the brain, gills and myotomal muscle (Iwama, 1998). Both epinephrine and cortisol have shown to have positive effects on glucose production (Randall and Perry, 1992; Iwama, 1998). Furthermore, exhaustive exercise, of which a rod-and-reel-angled tuna is certainly subjected to, may have significantly deleterious, and potentially lethal,

effects on blood acid-base status. These effects are of both metabolic (indicated by increasing blood lactate and decreasing blood bicarbonate) and respiratory (increasing pCO₂) origins, and effectively lower blood pH (see Perry et al., 1985; Wood et al., 1983; Wood, 1991; Kieffer, 2000; Skomal and Chase, 2002; Skomal, 2007; Suski et al., 2007; Mandelman and Skomal, 2009). Additionally, changes in hematological features and plasma ion concentrations are considered to be part of the secondary stress response (Barton, 2002).

Finally, tertiary responses to stress are those observable on a whole-organism scale or larger. Stress responses in fish are cumulative (Barton, 1997), and chronic stressors result in long-term energy repartitioning within a fish, which may have effects on growth, disease resistance and reproduction (Iwama, 1998; Barton, 1997; 2002). Clearly, these have implications for population and community structure (Iwama, 1998; Barton, 2002) in the wild, and for aquaculture operations, the successful establishment of a spawning broodstock population.

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