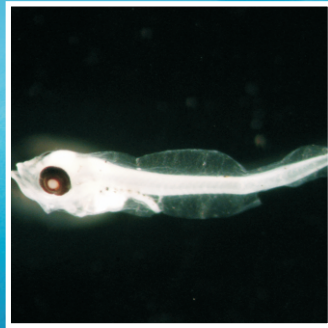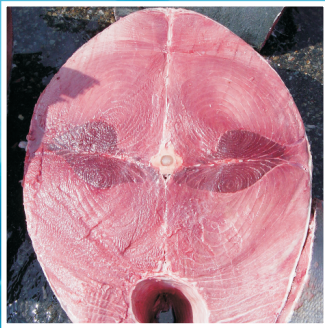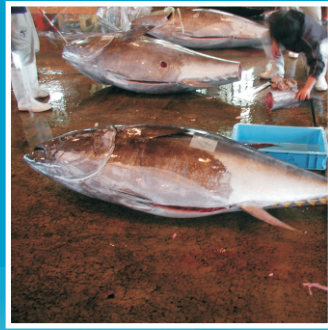# Biology and Ecology of Bluefin Tuna

Editors

Takashi Kitagawa
Shingo Kimura

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Biology and Ecology
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Bluefin Tuna

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Preface

Tuna are fascinating fishes that have been studied by fisheries scientists and fish ecologists for many years because of their high commercial value around the world. They are especially important in countries such as Japan, where tuna catches ranked the highest in the world in 2007, accounting for 14% (248,000 tons) of the world's total tuna catch. In addition, Japan is the largest tuna consumer in the world and is supplied with 473,000 tons of tuna (total amount of Japan's catches and imports; Fishery Agency 2009). Although Pacific (*Thunnus orientalis*), Atlantic (*T. thynnus*), and southern bluefin tuna (*T. maccoyii*) contribute relatively less in terms of total catch weight of the principal market tunas, their individual value is high because they are used for sashimi, which is a raw fish delicacy in Japan and increasingly in other countries (Majkowski 2007). Still fresh in our memories is the fact that a Pacific bluefin tuna caught off northeastern Japan fetched a record 155.4 million yen or about US$ 1.76 million in the first auction in January 2013 at Tokyo's Tsukiji fish market. The price for the 222-kg Pacific bluefin tuna beat the previous year's record of 56.49 million yen (Kitagawa 2013).

Bluefin tuna numbers have decreased by 80% or more since 1970 as a result of overfishing (Dalton 2005). In 2010, at the 15th Conference of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in Doha, Qatar, a proposal to list Atlantic bluefin tuna in Appendix I of CITES presented by Monaco was discussed and put to a vote. Although the proposed ban was voted down, the stock management of the bluefin tuna species is being further strengthened. Therefore, for proper stock management of the species, more detailed biological information is needed for bluefin tuna, such as about their ecology, distribution, and movements.

As represented by Sharp and Dizon (1978), Hochachka and Mommsen (1991), and Block and Stevens (2001), from whom we learned much, studies on tuna were intensively conducted from the latter part of the previous century to the beginning of this century. However, thanks to the development of various kinds of technologies of microelectronics, microchemistry, molecular genetic science, and computer science including mathematical modeling, studies on the biology and ecology of tuna have progressed greatly after 2000. On the other hand, a book about tuna has not been published since Block and Stevens (2001), and the previous books only examined 'tuna' in general, that is, fishes of the genus *Thunnus* and/or Scombridae. This book, therefore, focuses on the latest information on the biology and ecology of the three bluefin tuna species that are the Pacific, Atlantic, and southern bluefin tuna.

In the book, the phylogeny of the three bluefin tuna species is described in the first part, then basic ecological information such as early life history, age and growth, food habits, feeding strategy and predators are covered in the second part. Information related to migratory ecology, and important biological aspects of each of the three species, such as metabolism and energetics, swimming performance, schooling, visual physiology, and reproductive physiology are included in the third and fourth parts, respectively. In the last part, new research insights about a few kinds of mathematical models about bluefin tuna ecology and a technique for measuring swimming behavior of bluefin tuna are introduced. All the chapters of the book have been contributed by active scientists engaged in bluefin tuna research.

We sincerely hope that this book will contribute to a better understanding of the biology and ecology of bluefin tuna, and that undergraduate and graduate students who read this book will be encouraged to become bluefin tuna scientists who can contribute to further understanding of the biology and ecology of bluefin tuna.

Lastly, we would like to thank all the contributors to the book and Charles J. Farwell, Monterey Bay Aquarium, USA and Michael J. Miller, Nihon University, Japan, for their advice and encouragement to make this book. We are grateful for the editorial assistance provided by Itsumi Nakamura, Atmosphere and Ocean Research Institute, The University of Tokyo, Japan.

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Takashi Kitagawa
Shingo Kimura

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CHAPTER 1

Phylogeny of Bluefin Tuna Species

Nobuaki Suzuki[1,*] and *Seinen Chow*[2]

Phylogenetic Conflicts Among *Thunnus* Species: Morphology versus Molecular, Mitochondrial DNA versus Nuclear DNA

During the past half century, taxonomy, systematics and phylogenetic relationships among *Thunnus* tuna species have been investigated in depth by several eminent fish scientists. Before introducing the present view of phylogenetic relationships of *Thunnus* tuna species, we shall look at the history of tuna's systematics.

The systematic grouping and the phylogenetic implications were in complete harmony in the earlier decades but subsequently scientists made different postulates on the systematics of tuna based on detailed examinations for morphological characteristics. The comprehensive systematic studies of *Thunnus* were conducted from the 1960's and voluminous descriptions regarding measurements, meristics, coloration, osteological traits, viscera, vascular system and olfactory organ were made (Iwai et al. 1965; Nakamura 1965; Gibbs and Collette 1967). Comparing the 18 diagnostic characteristics, Gibbs and Collette (1967) recognized two groups of species in this genus; one the 'bluefin tuna group' consisted of *T. alalunga* (albacore), *T. thynnus* (northern bluefin tuna) and *T. maccoyii* (southern bluefin tuna) which were similar to each other in 14–16 diagnostic characters and the other the 'yellowfin tuna group' including *T. albacares* (yellowfin tuna), *T. atlanticus* (blackfin tuna) and *T. tonggol* (longtail tuna) which shared 15–16 characters. Gibbs and Collette also pointed out an intermediate state of diagnostic characters of *T. obesus* (bigeye tuna) between the two groups. These interpretations agreed with the intra-generic relationships presented by Iwai et al. (1965) and Nakamura (1965). Subsequently, Collette (1978) focused on internal morphology regarding adaptations for endothermy and considered that absence/presence of central heat exchanger within the bluefin tuna/yellowfin tuna

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groups, respectively, is a significant key to distinguish the two groups, and consequently interpreted the intermediate species of *T. obesus* as a member of the bluefin tuna group due to absence of this characteristic. Moreover, Collette (1978) hypothesized that the species of the bluefin tuna group have evolved from an ancestral tropical tuna and adapted into cooler waters due to the highly-developed lateral heat exchangers. However, there should be some concerns that the phylogenetic history was discussed based on adaptive characters.

Since 1980's, cladistic approach have been propagated to infer phylogenetic relationships of a wide variety of fishes. For scombroid fishes including the tunas, mackerels, billfishes and the relatives, several researches based on the approach have been conducted (Collette et al. 1984; Johnson 1986). Great accumulation in anatomical morphology of this group of fishes was strongly expected to find a trustable answer for the phylogenetic relationships. However, a robust phylogeny of scombroids was not able to be reconstructed and remained to be controversial because of conflicting and instability among resultant phylogenetic hypotheses (Carpenter et al. 1995). Since well-known morphology of scombroids could not even resolve the phylogenetic relationships among higher taxa than the genus level, it was certainly impossible to elucidate the interspecific relationships of the genus *Thunnus* in those days.

Next generation researches in tuna's phylogeny were brought by tremendous progress in biochemistry and molecular biology during the last decade of 20th century. Analytical techniques for allozymes (Elliott and Ward 1995) and mitochondrial DNA (mtDNA) (Bartlett and Davidson 1991; Block et al. 1993; Finnerty and Block 1995; Chow and Kishino 1995; Alvarado Bremer et al. 1997) were introduced to investigate the genetic relationships between *Thunnus* species and then genetic data different from morphological characters were collected. Based on the allozyme polymorphism analysis, Elliott and Ward (1995) reported genetic similarity among the five Pacific *Thunnus* species; Pacific northern bluefin (*T. t. orientalis*), southern bluefin, albacore, bigeye and yellowfin tunas, and implied the most divergent status of albacore. Finnerty and Block (1995) represented the novel phylogenetic hypothesis among scombroids including five tuna species (Atlantic northern bluefin, southern bluefin, albacore, bigeye and yellowfin tunas) inferred from partial nucleotide sequences of the mitochondrial cytochrome *b* gene, which indicated the sister relationship between albacore and the other monophyletic tunas and thus rejected monophyly of the bluefin tuna group previously supposed based on the morphology.

After the earlier molecular works, in order to illustrate the whole picture of *Thunnus* phylogeny and to conclude the taxonomy and systematics, Chow and Kishino (1995) and Alvarado Bremer et al. (1997) conducted comprehensive analyses including all of the seven species as well as the two northern bluefin subspecies. First, Chow and Kishino (1995) examined partial nucleotide sequences of the mitochondrial cytochrome *b* and ATPase genes in addition to Restriction Fragment Length Polymorphism (RFLP) of the internal transcribed spacer 1 (ITS1) in the nuclear rRNA gene family of all *Thunnus* species and then illustrated the comprehensive phylogeny. On the topology of the ATPase gene phylogeny, Pacific northern bluefin tuna (*T. t. orientalis*) was placed distant from the Atlantic subspecies (*T. t. thynnus*) but closely-related to albacore. Since the Atlantic northern bluefin and southern bluefin tunas were found to have mtDNA sequences very similar to species of yellowfin tuna group and not so similar

to albacore and bigeye tunas which were morphologically assigned to the bluefin tuna group, rejection for monophyly of the bluefin tuna group was corroborated as proposed by the earlier molecular results. However, Chow and Kishino (1995) also reported an important finding that no differentiation in nuclear genome was observed between the Atlantic and Pacific northern bluefin tunas, in which the both subspecies were observed to share the same RFLP profiles for ITS1 generated by eight restriction enzymes. Here, we can see a more complicated conflict between mitochondrial and nuclear results as well as that between morphology and molecular data. Chow and Kishino (1995) concluded that albacore was the earliest offshoot, followed by bigeye tuna in this genus, which is inconsistent with the phylogenetic relationships between these tuna species inferred from morphology, and suggested possible hybridization that the mtDNA from albacore has been incorporated into the Pacific population of northern bluefin tuna and has extensively displaced the original mtDNA. Subsequently Alvarado Bremer et al. (1997) examined the rapidly evolving control region of mtDNA and presented the phylogenetic relationships almost consistent with Chow and Kishino (1995) except that bigeye tuna was identified as the sister species of the yellowfin tuna group. The authors concluded that, since the Atlantic and Pacific northern bluefin tunas were more divergent from each other than the average distance separating most species-pairs within the genus, a re-examination of their status as the Pacific subspecies of *T. thunnus* was warranted. After taking into account both molecular data introduced above and morphological differences (shape of the dorsal wall of the body cavity in large specimens and numbers of gill rakers), Collette (1999) and Collette et al. (2001) advocated separation of the northern bluefin tuna into the Atlantic species, *T. thynnus*, and the Pacific species, *T. orientalis* (Table 1.1).

Recently in order to elucidate the enigma regarding conflict between mitochondrial and nuclear phylogenies and to conclude phylogenetic relationships of *Thunnus* species, Chow et al. (2006) investigated the tuna's ITS1 nucleotide sequences with considering intraspecific variation of all of *Thunnus* species and with comparing the result to the updated phylogeny from mtDNA sequences. On both the ITS1 and the mtDNA phylogenies, the yellowfin tuna group was inferred as monophyletic, and southern bluefin and bigeye tunas showed a closer affinity to this tropical tuna group than to the northern bluefin tunas and albacore at least on the ITS1 phylogeny (Fig. 1.1). Therefore,

Table 1.1. Synopsis of current classification of *Thunnus* species.

Genus *Thunnus* South, 1845	
(Subgenus *Thunnus* South, 1845)	
Thunnus alalunga (Bonnaterre, 1788)	Albacore
Thunnus maccoyii (Castlenau, 1872)	Southern bluefin tuna
Thunnus obesus (Lowe, 1839)	Bigeye tuna
Thunnus thynnus (Linnaeus, 1758)	Atlantic bluefin tuna
Thunnus orientalis (Temminck and Schlegel, 1844)	Pacific bluefin tuna
(Subgenus *Neothunnus* Kishinouye, 1923)	
Thunnus albacares (Bonnaterre, 1788)	Yellowfin tuna
Thunnus atlanticus (Lesson, 1831)	Blackfin tuna
Thunnus tonggol (Bleeker, 1851)	Longtail tuna

This classification follows Collette (1999) and Collette et al. (2001).
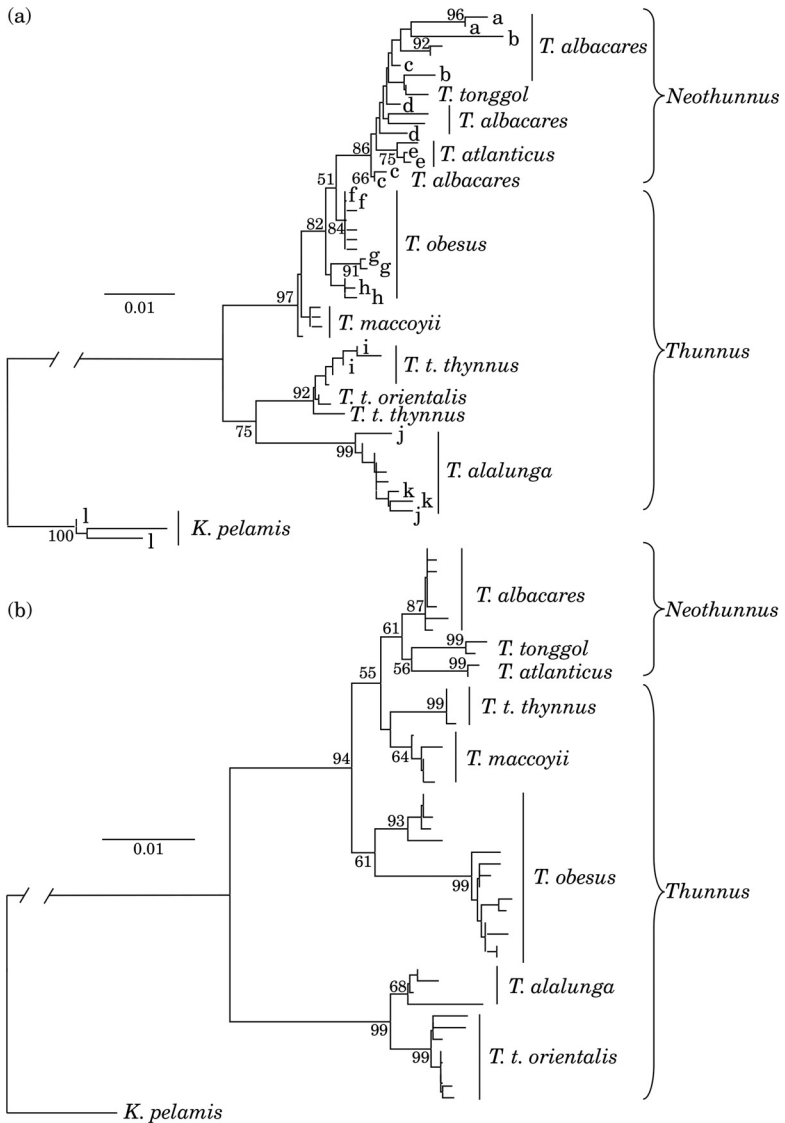
Figure 1.1. Neighbor-joining phylogenetic trees based on (a) nuclear rRNA ITS1 and (b) mtDNA AT sequence data (Chow et al. 2006). Bootstrap values >50% (out of 1000 replecates) are shown at the nodes.

although the relationships within the yellowfin group were still uncertain, the group should be existent based on similarities either from morphological and ecological characteristics or from nuclear and mitochondrial DNAs. On the other hand, the other morphological grouping, the bluefin tuna group, appeared to be questionable because the ITS1 and mtDNA topologies showing intermittent speciation of the bluefin tuna group members contradicted morphological subdivision of the *Thunnus* species. The resultant ITS1 phylogeny also provided remarkable similarity among the sequences
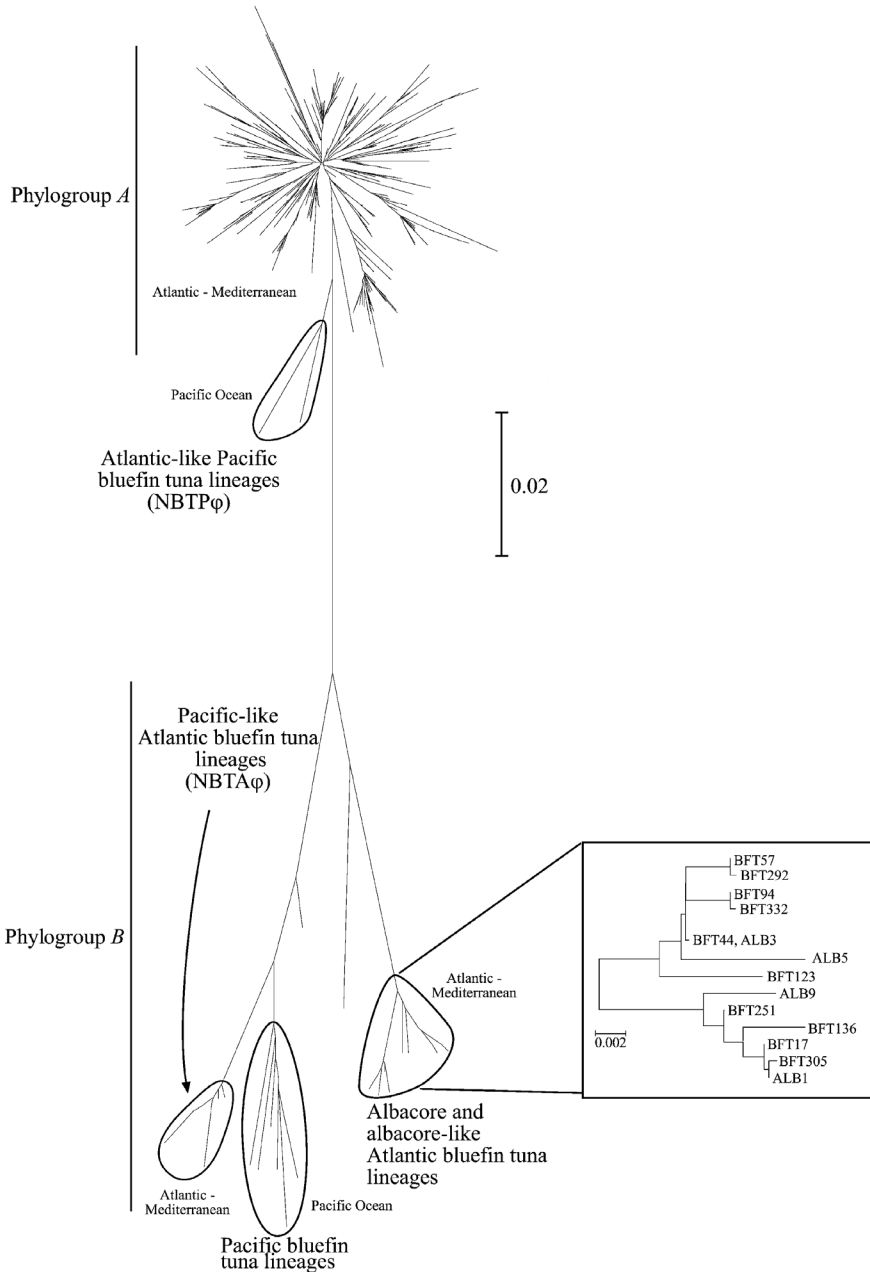
Figure 1.2. Unrooted neighbor-joining trees showing the relationships of mtDNA control region sequences for 334 Atlantic bluefin tuna haplotypes and for reference sequences of seven Pacific bluefin tuna and four albacore. Inset: subtree with albacore and albacore-like Atlantic bluefin tuna lineages. Alvarado Bremer et al. (2005) was modified.

from the Atlantic and Pacific northern bluefin tunas (Fig. 1.1a), which corresponded to the range of intraspecific variation, like as the identical RFLP profiles proposed by Chow and Kishino (1995). While the sister relationships among northern bluefin tunas and albacore were illustrated on the ITS1 phylogeny, the Pacific northern bluefin tuna alone was closely related with albacore on the mitochondrial phylogeny (Fig. 1.1b). The clustering the Pacific northern bluefin tuna with albacore in the mtDNA phylogeny may not be a simple consequence of the mitochondrial introgression from albacore to Pacific bluefin tuna proposed by Chow and Kishino (1995), but suggested an alternative mitochondrial introgression between the ancestral lineage of northern bluefin tuna and a species in the stem gene pool which subsequently led to the southern bluefin tuna, bigeye tuna and tropical tunas lineages. Consequently Chow et al. (2006) concluded the specific status of the two northern bluefin tunas would remain unresolved until more data from the nuclear genome become available.

As described above, a large amount of research focused on phylogenetic relationships and systematics of *Thunnus* tuna species have been performed during the last half century. Morphological, ecological and molecular data have also been accumulated continuously. The phylogeny that we can see, however, has been vague yet and some questions remain unresolved. Regarding the bluefin tuna species, it is sure that the most important issue is of phylogenetic relationship between Atlantic and Pacific northern bluefin tunas. Next we will focus further on inter- and intra-specific phylogeny of the two northern bluefin tunas.

Relationships Among Atlantic and Pacific Northern Bluefin Tunas

Alvarado Bremer et al. (2005) carried out comparative phylogeographic and historical demographic analyses for both Atlantic bluefin tuna and swordfish (*Xiphias gladius*) to clarify the complex phylogenetic signals in the North Atlantic-Mediterranean region. In the analysis for Atlantic bluefin tuna, nucleotide sequences of the mtDNA control region from the 607 individuals were investigated with those from Pacific bluefin tuna and albacore. The resultant mtDNA phylogeny obviously indicated reciprocal and monophyletic sister clusters of either Atlantic bluefin tuna or Pacific bluefin tuna in the Pacific and in the Atlantic, respectively. Definitely the Atlantic-like mtDNA lineage among Pacific bluefin tuna was reported by Chow and Kishino (1995) and Takeyama et al. (2001), while the Pacific-like mtDNA lineage among Atlantic bluefin tuna was found by Alvarado Bremer et al. (1999). These results should be interpreted as either Atlantic or Pacific bluefin tunas consisting of polyphyletic origins of mtDNA. The nucleotide sequence divergence between the Pacific bluefin tuna mtDNA and the Pacific-like Atlantic bluefin tuna mtDNA was estimated to be approximately 4.7% in the mtDNA control region, which was almost consistent with the reciprocal comparison between the Atlantic bluefin tuna mtDNA and the Atlantic-like Pacific bluefin tuna mtDNA (4.8%). Alvarado Bremer et al. (2005) attempted to propose a possible scenario to explain the reciprocal relationships between the Atlantic and the Pacific bluefin tunas, in which the common ancestors were sympatric until being separated by the rise of the Isthmus of Panama, about 3.0–3.5 million years ago and then gene flow between them have been prevented for a long time. According to the

scenario, however, mutation rate for the mtDNA control region was estimated between 1.3–1.6% substitutions per million years, which would be too slow to compare the previous estimates for other teleosts. Therefore the authors explored an alternative calibration for the mtDNA control region in teleosts and adopted the rate of 4.9–5.7% substitutions per million years according to Donaldson and Wilson (1999) and Tringali et al. (1999). Using this calibration, cladogenesis splitting the Atlantic bluefin tuna mtDNA and the Atlantic-like Pacific bluefin tuna mtDNA was estimated to occur just prior to the glacial events about 980–840 thousand years ago, followed by population size expansion in the Atlantic Ocean during a warm period that occurred approximately 450–390 thousand years ago. It should be a likely estimation at present that induced from molecular phylogeny, historical demography and geographic information regarding the Atlantic bluefin tuna and its relative lineages.

When we consider phylogenetic relationships between Atlantic and Pacific bluefin tunas, one more confusing issue emerges from their evolutionary histories. That is possible introgressions between the northern bluefin tunas and albacore. First Chow and Kishino (1995) described closer relationships between Pacific bluefin tuna and albacore mtDNAs than that of Atlantic bluefin tuna in contrast with no differentiation regarding electropholetic patterns of nuclear ITS1 RFLP between Atlantic and Pacific bluefin tunas. Since Atlantic bluefin and southern bluefin tunas were also found to have similar mtDNA sequences, Chow and Kishino (1995) proposed the hypothesis that albacore mtDNA has been introgressed into the Pacific bluefin tuna and that three bluefin tuna species should be closely-related to each other. Subsequently Chow et al. (2006) presented an alternative hypothesis regarding albacore mtDNA introgression because of clustering the two northern bluefin tunas with albacore in ITS1 sequence phylogeny, which has been uncertain yet but possibly occurred between the ancestral lineage of northern bluefin tunas and a species in the stem gene pool which subsequently led to the southern bluefin tuna, bigeye tuna and tropical tunas lineages. In Atlantic bluefin tuna, however, a different case of close relationships between Atlantic bluefin tuna and albacore mtDNAs was found by Alvarado Bremer et al. (2005). About 3.3% of Atlantic bluefin tuna mtDNA sequences were interspersed among the albacore mtDNA cluster in the phylogeny and also found to include one shared sequence. This implies a recent hybridization event between Atlantic bluefin tuna and albacore and may support an introgression hypothesis causing confusing phylogenetic relationships among bluefin tuna species as well as albacore.

In contrast to the confusing relationships in mtDNA lineages among Pacific and Atlantic bluefin tunas together with albacore, clear geographic population subdivision among Atlantic bluefin tuna was detected using nuclear DNA markers. Albaina et al. (2013) discovered a total of 616 Single Nucleotide Polymorphisms (SNPs) from 54 PCR amplicons of nuclear DNA among 35 fish samples of albacore. Of all the SNPs, 128 SNPs were attempted in cross-species genotyping for Atlantic bluefin tuna, of which 17 SNPs corresponding to 15 markers (13 SNPs and two haplotype blocks) were validated for PCR amplification, data quality and Mendelian population genetic assumptions. Despite a small number of markers, these 15 markers succeeded to detect significant overall differentiation among 107 individuals from three geographic samples in the northwestern Atlantic, northeastern Atlantic (Bay of Biscay) and Mediterranean

(Balearic Sea) (P < 0.05). Individual clustering analysis illustrated that the three samples most likely represented two genetically distinguishable populations, that is, northwestern Atlantic or northeastern Atlantic plus Mediterranean (Fig. 1.3), that corresponded to the two major spawning grounds of Atlantic bluefin tuna. In contrast that the young-of-the-year samples from northwestern Atlantic and Mediterranean should be represented for each spawning group, the northeastern Atlantic sample composed mixed age classes from juveniles to adults foraging in the Bay of Biscay. Therefore the SNP analysis indicated the Mediterranean origins of foraging individuals in the Bay of Biscay.

The bluefin tuna phylogeny and their evolutionary history with the close relatives have remained to be ambiguous as described above. However, new effective approaches such as the SNP markers by Albaina et al. (2013) are appearing in fish researches. Recently, genomic approach is becoming common not only in medical sciences for human health but also in natural sciences for wild organisms. Next, some brand-new genomic studies targeted at *Thunnus* tuna species are introduced. A variety of genomic approaches will be forthcoming powerful tools for improving population genetics, phylogenetic systematics and evolutionary ecology, and may provide breakthroughs to disentangle confusing relationships among bluefin tuna species.
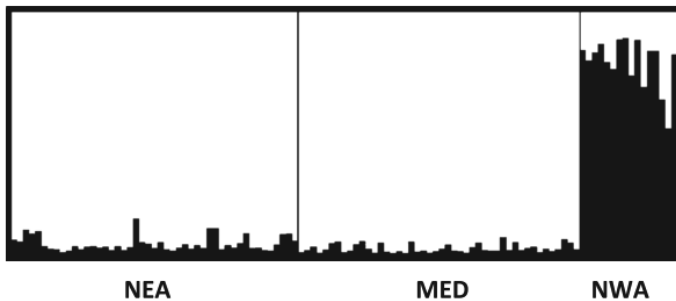
Figure 1.3. Individual clustering analysis of 107 Atlantic bluefin tuna for 17 single nucleotide polymorphisms (SNPs) on 15 independent DNA fragments. Each vertical bar represents an individual, and sampling locations are separated by vertical black lines. The color proportions of each bar correspond to the individual's estimated membership fractions to each of the clusters (cluster membership coefficient) (Albaina et al. 2013).

An Epoch in Tuna Genomics

In the last decade, nucleotide sequencing capability has been dramatically progressing due to spread of multi-capillary sequencers or to the appearance of Next-Generation Sequencers (NGS), which necessitate rapid development of bioinformatic technology to analyze a huge amount of sequence reads generated from NGS. This situation allows us to perform much larger-scale analyses beyond our earlier expectations.

The evolutionary origin of the family Scombridae including *Thunnus* tuna species has been a longtime issue despite long-term arguments based on morphological and molecular data (Collette et al. 1984; Johnson 1986; Block et al. 1993; Orrell et al. 2006). As a new approach to address the issue, Miya et al. (2013) collected more

than 10,000 sequences of percomorph fishes from international DNA database and bioinformatically sorted into nine homologous protein-coding (six mitochondrial and three nuclear) genes, which finally comprised 5,367 species across 1,558 genera and 215 families (87% of total percomorph family diversity). A whole result from each phylogeny of the individual genes suggested that 15 perciform families formed the least monophyletic group that contains all core members of the classical scombroid families (Gempylidae, Trichiuridae, Scombridae). According to the bioinformatic result, Miya et al. (2013) sampled a total of 124 complete mitochondrial genomic sequences, which consisted of 56 species from those 15 families (in group) and 68 out group species including representative pelagic percomorphs, with generating original sequences from 37 in group species and from 17 out group species. Large data sets including more than 10,000 mitochondrial nucleotides from 12 protein-coding genes, 22 tRNA genes and 2 rRNA genes were produced and then analyzed by phylogenetic maximum likelihood estimation. The result from the mitogenomic analysis supported a monophyly of the 15 families noticed above with 100% bootstrap probability and the family Scombridae also indicated to form a robust monophyletic group with strong statistical support (Fig. 1.4). Additionally Miya et al. (2013) proposed the time-calibrated phylogeny and the ecological mapping of specific habitat depth on the topology, consequently suggesting a possible origin of scombrids from a deep-water ancestor and subsequent radiation following the Cretaceous-Paleogene mass extinction including large predatory epipelagic fishes. In their study, a new approach that combined an unprecedented scale of both partial and complete mitochondrial genomic data with bioinformatic technology was able to illustrate the phylogenetic framework and the evolutionary scenario regarding scombrids clearly. Although phylogenetic relationships among and within tuna species were not focused in this study, large-scale mitogenomic analyses with many more samples covering a geographic range of each species might be able to disentangle mitochondrial phylogenetic uncertainty of *Thunnus*.

Another innovative work was brought by an application of NGS. Nakamura et al. (2013) reported the complete genome sequences of a male of Pacific bluefin tuna caught as a young-of-the-year juvenile off the Pacific coast of Japan and reared for about three years, in which genetic and evolutionary basis of optic adaptation of tuna was elucidated. A whole-genome shotgun sequencing and subsequent bioinformatic assembling provided a total of 740.3 Mb sequences consisting of 192,169 contigs (>500 bp) and 16,802 scaffolds (> 2 kb), corresponding to 92.5% of the estimated genome size (~800 Mb). Additional NGS sequencing was carried out for total cDNA from another adult female reared at age five, and mapping the cDNA sequences as well as Expressed Sequence Tags (ESTs) from the Atlantic bluefin tuna on the genome of Pacific bluefin tuna predicted more than 26,000 protein-coding gene candidates. Pairwise comparison with known genome sequences from six teleosts (cod, medaka, zebrafish, stickleback, greenpuffer, fugu) found out 6,170 conserved genes in the Pacific bluefin tuna genome. Among the genes, 10 visual pigment genes were identified and compared with fish opsin sequences deposited in the DNA databases. Based on the multiple alignments, Nakamura et al. (2013) found amino acid substitutions at spectral tuning sites of rhodopsin and four green-sensitive opsin genes (Table 1.2) which involved light sensitivity and may be tuned to blue light. It is likely that these substitutions contribute to spectral tuning to the offshore environment, for better
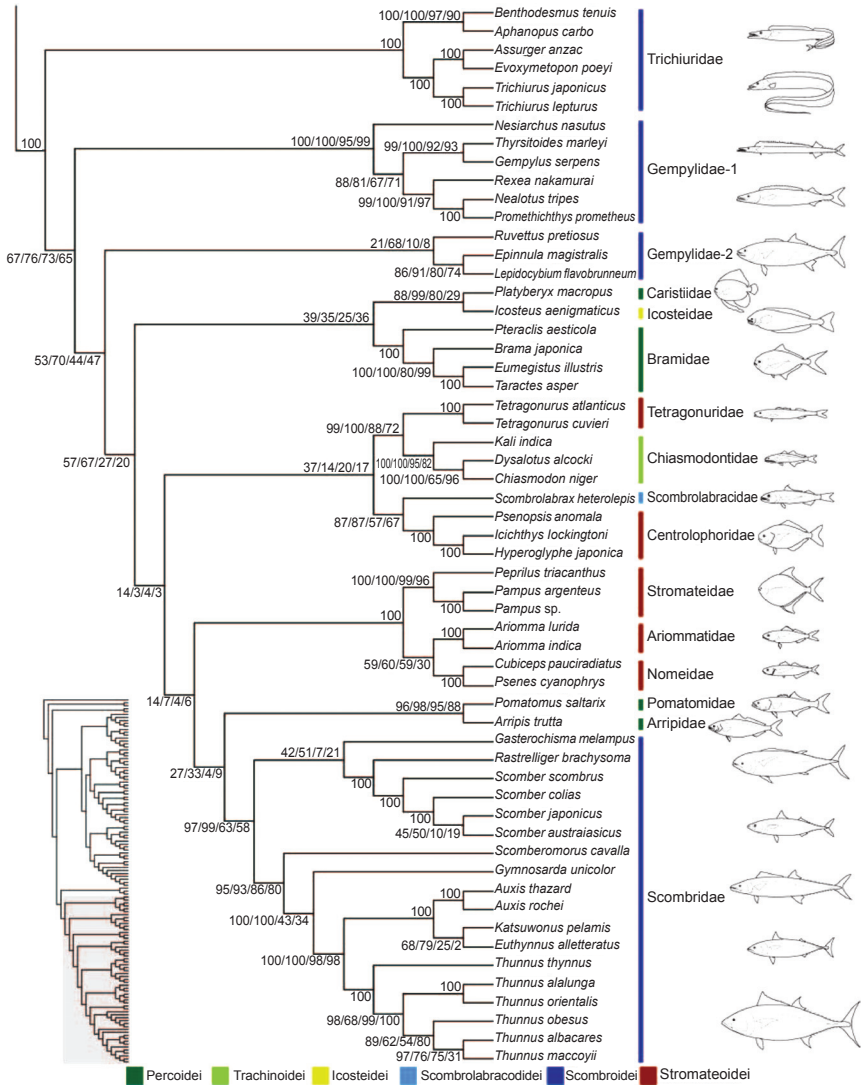
Figure 1.4. The best-scoring maximum likelihood tree based on whole mitogenome sequences. Bootstrap values >50% (out of 1000 replecates) are shown at the nodes. Miya et al. (2013) was modified.

Table 1.2. Representative amino acid sites involved in the light sensitivity of rhodopsin in comparison with seven teleosts.

Tuning site	zebrafish	cod	medaka	greenpuffer	fugu	stickleback	tuna
83	D	N	D	N	D	D	D
122	E	E	E	E	E	E	Q
261	F	F	F	F	F	F	F
292	A	A	A	A	A	A	A

cognition for bluish contrasts, and consequently to effective detection of prey in the bluish ocean. Phylogenetic analysis illustrated that the amino acid substitution of rhodopsin was widely conserved among the suborder Scombroidei containing the family Scombridae (Fig. 1.5), suggesting that the molecular evolutionary change
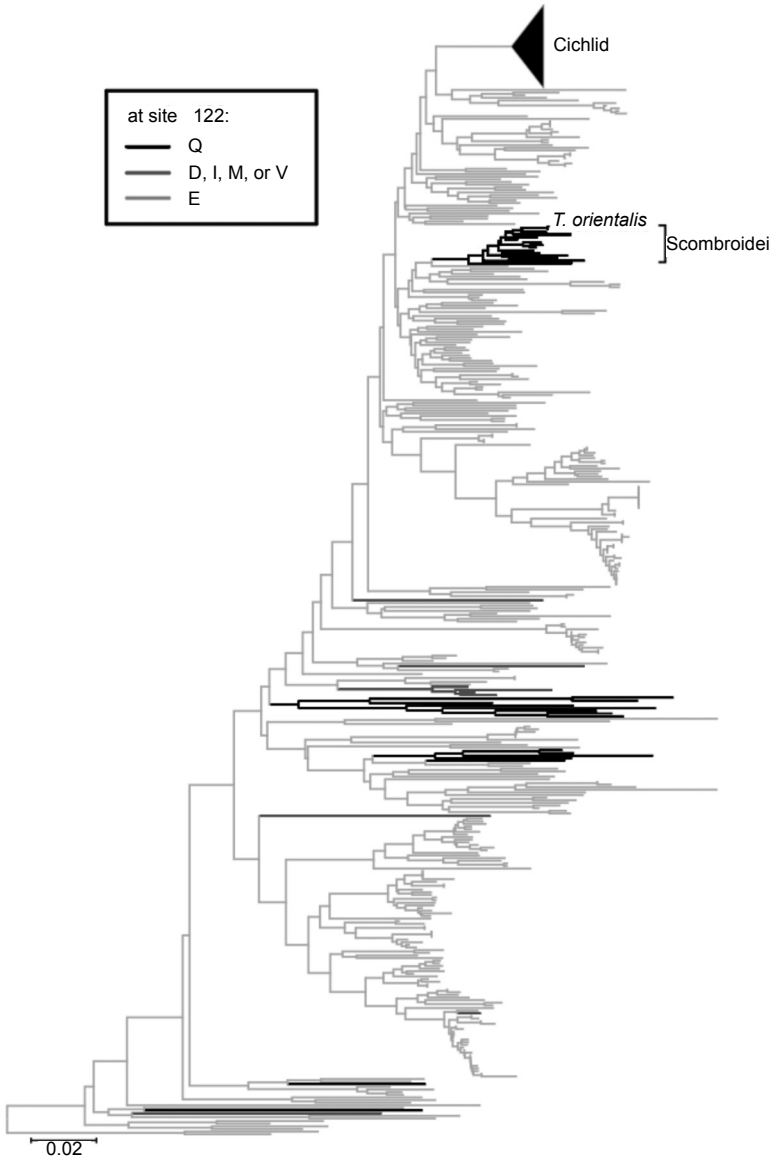
Figure 1.5. A neighbor-joining tree of fish rhodopsin sequences. A total of 557 nucleotide sequences including that for tuna (accession no. BAG14281) were collected from the GenBank database. The species that have glutamine (Q) at site 122 are colored in blue. For site 122, the species with aspartic acid (D), isoleucine (I), methionine (M), or valine (V) are colored in red, and those with glutamic acid (E) are colored in gray. Nakamura et al. (2013) was modified.

occurred in an ancestor of this lineage. Phylogenetic analysis also suggested that gene conversions have occurred in each of the blue- and green-sensitive gene loci in a short period. Those genetic recombinations may have facilitated adaptation to the offshore environment. Further genomic information from a wide range of pelagic fishes including tunas will provide significant clues for comprehensive understanding of biodiversity and adaptation of *Thunnus*.

In this chapter we followed the progress of researches about taxonomy, systematics and phylogenetic relationships for *Thunnus* species mainly focusing on bluefin tunas during the last half century, recognizing that many issues remain such as conflicts among morphology, mitochondrial DNA and nuclear genome. And finally we reached two large-scale genomic researches introduced earlier. Genome-wide sequence analysis was performed only in the Pacific bluefin tuna in the genus *Thunnus*, but the up-to-date genomic data provided a new interesting and detailed insight for tuna evolution and adaptation. As noted by Albaina et al. (2013), the NGS technology will undoubtedly realize a wide variety of genome-wide examinations in non-model organisms in the near future. Using NGS approaches targeted at total genomes as well as mitochondrial genomes, further applications for more samples of *Thunnus* species are largely expected to disentangle the phylogenetic uncertainty.

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II. Basic Ecology of Bluefin Tuna

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CHAPTER 2

Early Life History

Yosuke Tanaka[1,*] and *Nobuaki Suzuki*[2]

Introduction

Many studies concerning the early life history of Pacific bluefin tuna *Thunnus orientalis* have been conducted since the 1950s in Japan. Generally, studies of the early life history of marine fishes are composed of two approaches; field surveys and rearing experiments. At the beginning, the studies conducted for the early life history of Pacific bluefin tuna were the former. From the 1950s to the 1980s, large scale surveys of field sampling of the larvae were conducted around the spawning grounds formed in the northwestern Pacific Ocean. A lot of information about the species identification and the distribution of the larvae were accumulated through these surveys. Thereafter, in the 2000s studies concerning the growth and development of larvae and survival mechanisms in relation to those have been conducted using various analysis methods.

On the other hand, studies on technique development for artificial mass culture have been promoted from the 1990s aimed at promoting aquaculture and stock enhancement in Japan. The most remarkable work in this process is the life completion of Pacific bluefin tuna under aquaculture conditions at Kinki University accomplished in 2002 (Sawada et al. 2005). In the processes of the technique development of mass cultures, various rearing experiments have revealed the ecological, physiological and behavioral traits of the early life stages of Pacific bluefin tuna, which are difficult to be researched based on field captured specimens.

Thus, recent studies on the early life history of Pacific bluefin tuna have been conducted by field surveys and rearing experiments. Since Pacific bluefin tuna is the only species to be reared throughout their whole life stages among other tuna species, we focus on the early life history of Pacific bluefin tuna. In this chapter, we review the eco-physiological and behavioral traits and the survival mechanisms in the early life history of Pacific bluefin tuna, revealed by both field surveys and rearing experiments.

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Distribution of Larvae and Juveniles of Pacific Bluefin Tuna

Spatiotemporal extent of the larval distribution in the Pacific Ocean

Research surveys to analyze the geographical distribution of Pacific bluefin tuna larvae can be categorized into three generations, which have been carried out for over more than a half of century by Japanese scientists. The first generation of surveys (1956–1989) was summarized by Nishikawa et al. (1985) and Yonemori (1989), focused on understanding the spawning grounds and periods based on the extent of larval distribution in the northwestern Pacific Ocean. First, Ueyanagi (1969) threw light upon the geographical patterns of the larval distributions of the tuna species within the Indo-Pacific oceans by presenting charts where sampling sites and catch records of larvae of albacore, yellowfin, bigeye, skipjack, Pacific bluefin and southern bluefin tunas were given. Regarding the Pacific bluefin tuna, a relatively restricted larval distribution was found around the Kuroshio Current and its counter current regions from the south of Japan to the east of Taiwan (see Appendix Fig. 8 in Ueyanagi 1969). Next, Nishikawa et al. (1978) and Nishikawa et al. (1985) attempted to compile all of the data collected by both research vessels as well as local government vessels during the past large-scale sampling program, to calculate the Catch Per Unit Effort (CPUE) in terms of the number of larvae per 1,000 m³, and to illustrate either annual or quarterly global distributions of larval densities of various scombrid fishes. Complete data analyzed in Nishikawa et al. (1985) was compiled from 63,017 tows of 1.4 m or 2.0 m diameter plankton net conducted from 1956 to 1981. The resultant chart succeeded to enable a more detailed distribution of Pacific bluefin tuna larvae than that by Ueyanagi (1969), identifying the putative spawning area ranging from the Bashi Strait to the southern coast of central Japan with the spawning season mainly from April to June. Additionally Nishikawa et al. (1985) referred to the occurrence of larvae from the Sea of Japan during early August in the text despite no plot being given on the chart, and then discussed the strong 'homing' nature of this species based on the limited spawning area compared to the Pacific-wide adult distribution. In order to clarify whether this species does in fact show 'homing' behavior, it is necessary to consider the spawning ecology of this species.

Following the earliest works that had provided basic information on spawning grounds of wild Pacific bluefin tuna, large-scale surveys called 'Marine Ranching Project' were conducted from 1980 to 1989 with a preliminary survey in 1979. Yonemori (1989) summarized the 10-year project, clearly indicating two geographically-isolated spawning grounds, the Nansei area and the Sea of Japan, as well as the spatiotemporal extent of the larval distribution (Fig. 2.1). Through the project, 928 surface trawl tows in surrounding waters of the Nansei Islands, 152 tows in the Pacific offshore waters of the Japanese main island, Honshu, and 138 tows in the Sea of Japan were carried out using 2 m diameter plankton net, consequently more than 10,000 individuals of Pacific bluefin tuna larvae were sampled and examined. Although the Nansei area had relatively higher CPUEs than those either in the Pacific offshore waters or in the Sea of Japan, sampling sites with high CPUEs varied among year to year fluctuations within the areas. Therefore the Nansei area should be considered as the main ground for spawning of the Pacific bluefin tuna but the spawning behavior
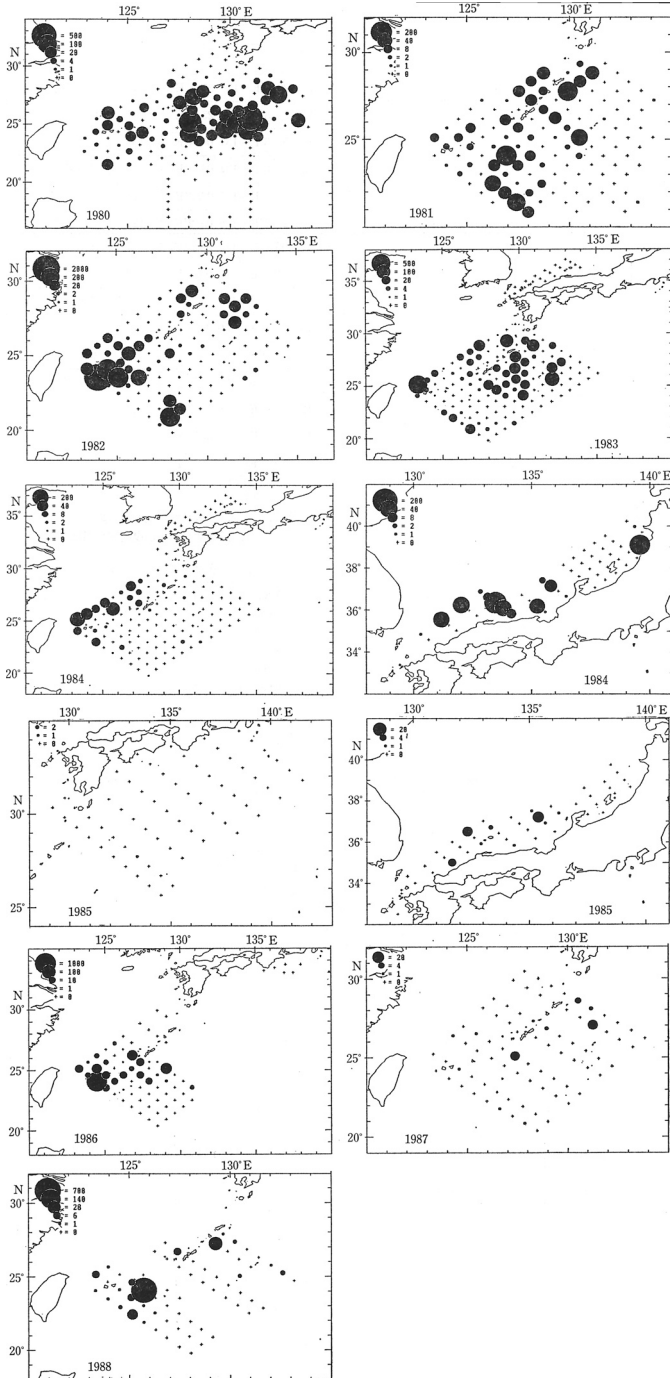
Figure 2.1. Survey area, sampling stations and annual catches of larvae and early stage of juveniles of Pacific bluefin tuna from 1980 to 1988 (Yonemori 1989).

may be adaptively changeable. The larvae occurred at 23.5 to 29.5ºC of Sea Surface Temperature (SST), most frequently at 25 to 27ºC (Yonemori 1989), and this SST can therefore be considered as the most appropriate condition at the main spawning ground. In addition, since the larvae tended to occur around the Yaeyama Islands, the western part of the Nansei area, and near the offshore edge of the Kuroshio Current including the counter current region, and not occurring on the continental shelf of the East China Sea, Yonemori (1989) concluded that the Pacific bluefin tuna might spawn more actively in the counter current region with oceanographic eddies than in the main stream region of the Kuroshio Current. Lower CPUEs observed in the Pacific offshore waters of Honshu and in the Sea of Japan, in contrast to the Nansei area, also provided evidence of spawning events in both areas. These findings succeeded to illustrate details of the spatiotemporal extent of the spawning grounds of Pacific bluefin tuna; middle May to late June in the Nansei area, late June to early July in the Pacific offshore waters of Honshu, and late July to mid August in the Sea of Japan. In addition, the spawning in the Sea of Japan was considered to occur every year based on larval catches in 1984 and 1985, alternatively the spawning in the Pacific offshore waters of Honshu appeared to be more restricted. As noted above, a whole picture of the distribution of the Pacific bluefin tuna larvae was drawn in the first generation of surveys, but environmental details providing the basis for better understand the larval distribution were still lacking.

Since there was a suspension of surveys following the Marine Ranching Project, we needed to wait subsequent progress until the beginning the second generation of surveys from 2004 to 2010. In the second period, two types of surveys were conducted focusing on high density larval distributions (patches) of the larvae; radio buoy tracking (2004–2008) and environmental adaptive sampling (2007–2010). These surveys were designed to develop new strategies for both larval sampling and oceanographic observations in waters of the major spawning ground, the Nansei area. Radio buoy tracking accompanying oceanographic observations by Conductivity Temperature Depth (CTD) profiling and Acoustic Doppler Current Profiler (ADCP) represented detailed distribution of larval patches in relation to mesoscale eddies successfully as well as the vertical and horizontal extents of the patch structure, larval growth and natural mortality (Satoh et al. 2008; Satoh 2010; Satoh et al. 2013). Satoh (2010) described patches entrained in mesoscale eddies (~100 to 500 km diameter), and therefore considered that the positional relationship between spawning events and mesoscale eddies was important for the recruitment process of the larvae (Fig. 2.2). Similarly adaptive sampling trials adjusting to oceanographic conditions in the Nansei area detected occurrence of larval patches around mesoscale eddies and succeeded to converge the survey area to monitor year-to-year fluctuations in amounts of larval catches irrespective of oceanographic conditions (Suzuki et al. 2014) (Fig. 2.3). Marked collections of Pacific bluefin tuna larvae were made at sampling sites located between 24º and 26º N along a 126º E line throughout the four-year surveys, which seemed to be influenced by mesoscale eddies based on sea surface level structure and local currents resulting from bottom topography. Therefore the second generation of surveys provided fragmentary information of relationships between the larval distribution and oceanographic condition but enabled development of the next scheme for further surveys focusing on fluctuations in biological and environmental factors.
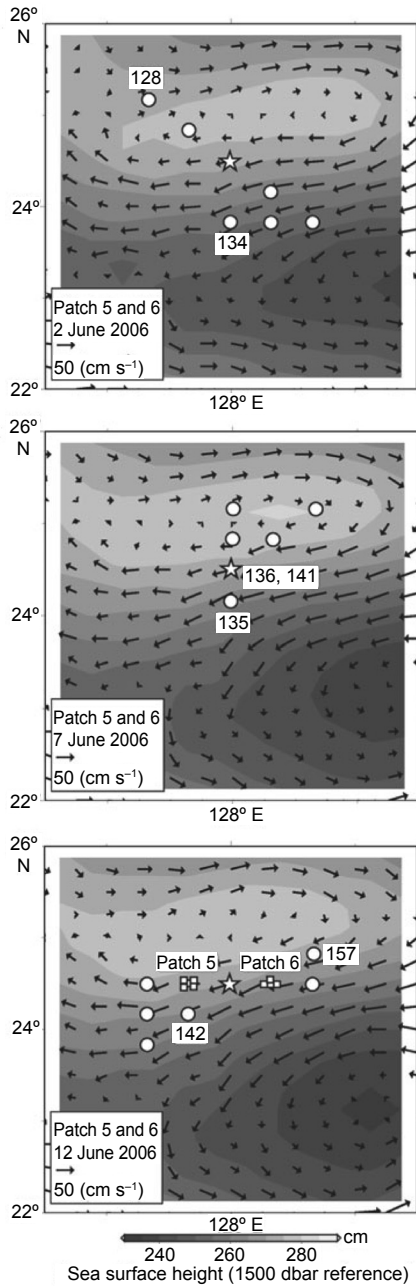
Figure 2.2. Sea surface height, sea surface currents and sampling stations within the Nansei area in June 2006. Open circles with/without numbers show sampling stations. Stars indicate stations at the center of the schematic observation. Patches 5 and 6 were detected at the stations 146 and 151, respectively. Modified from Satoh (2010).
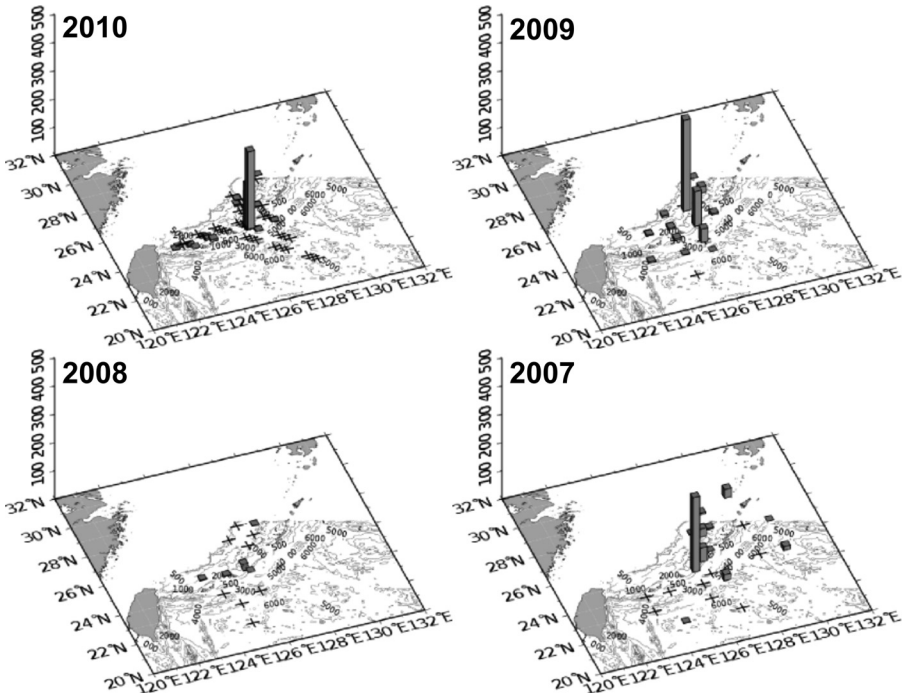
Figure 2.3. Horizontal plots of annual catches of Pacific bluefin larvae in the Nansei area from 2007 to 2010. Bars indicate numbers of larvae and '+' indicates no catch at a given station (Suzuki et al. 2014).

The most recent surveys (2011-), the third generation, have been continuing to evaluate the spatiotemporal extent of the larval distribution in Japanese waters including the two spawning grounds and to identify both spawning spots and their surrounding environments. Possible spawning spots, where the spawning events actually occurred, were estimated based on the catch of the larvae using back calculations on an oceanographic numerical model, though much less larval collections in the Sea of Japan may provide rough estimates of the spots in the area. The calculation also can provide possible trajectory of advection history and oceanographic conditions experienced by the larvae. This new approach that consists of biological sampling including the Pacific bluefin tuna larvae and other plankton species, oceanographic observations, and back calculations on the numerical model is anticipated to clarify the spawning ecology and larval survival processes of the Pacific bluefin tuna in the near future.

Analytical and theoretical approaches for the larval distribution

As noted above, basic information on the distribution and environmental conditions of the larvae have been accumulated over about a half of the century, but analytical and/or theoretical works to validate the favorable conditions for the larvae are strongly required for a better understanding larval distribution, spawning and reproductive ecology, and the early life history of the wild Pacific bluefin tuna. In the Atlantic congener, *T. thynnus*, many more studies to analyze field data using statistics and

numerical modeling have been energetically performed for both spawning grounds, the Gulf of Mexico (Teo et al. 2007; Muhling et al. 2010; Lindo-Atichati et al. 2012; Muhling et al. 2013) and the Mediterranean Sea (Alemany et al. 2010; Mariani et al. 2010; Muhling et al. 2013; Rodriguez et al. 2013). In contrast, in the Pacific bluefin tuna larvae, few studies have been made except for the modeling approaches on larval and juvenile dispersal processes by Kitagawa et al. (2010) and to evaluate possible impacts of the global warming by Kimura (2010). Regarding the larval transporting processes and the evolutionary interpretation on having both restricted spawning area and season compared to other tropical tuna species, Kitagawa et al. (2010) presented clear evidence based on numerical simulations that reproduction of larvae around the Nansei area, not in the Pacific offshore waters of Honshu, should enable effective recruitment of the Pacific bluefin tuna larvae into the nursery area located in Japanese coastal waters, irrespective of annual fluctuations in oceanographic conditions (Fig. 2.4). According to Kitagawa et al. (2010), restricted spawning area and season of Pacific bluefin tuna could be explained as a consequence to maximize success of larval recruitment into optimal thermal conditions for better growth and survival with low energetic costs rather than as a preferable environment for spawning adults. This is considered to be strongly related to the evolutionary history of the Pacific bluefin tuna and seems to be largely consistent with a hypothetical scenario that the period from larval to very early juvenile stages is critical for density-dependent controls operating on the population dynamics. Theoretically possible hypotheses of evolutionary mechanisms that had generated restricted spawning grounds and limited larval distribution in bluefin tuna species have been proposed by Bakun (2006; 2013), based on strongly convergent environments around energetically forced ocean eddies and interactions between larvae and their predators. Further analytical as well as modeling studies based on methods such as described by Kitagawa et al. (2010) are likely to present some kind of empirical evidence for ecological and evolutionary hypotheses of recruitment processes of the Pacific bluefin tuna.

Distribution of juvenile Pacific bluefin tuna

Juvenile Pacific bluefin tuna (< 50 cm Fork Length, FL) are distributed around the coastal area of Japan and are caught by troll and/or purse seine fisheries in the Pacific Ocean and in the Sea of Japan, part of which are used as seedlings for aquaculture. Fisheries-based information on the juvenile distribution, therefore, has been sufficiently accumulated but scientific research surveys have been lacking with the fisheries due to the absence of appropriate sampling gears and skills. To capture sufficient numbers of tuna species juveniles, midwater trawl net with a large mouth opening capable of high speed towing was developed and adopted for research surveys targeting tuna juveniles (Tanabe and Niu 1998; Itoh et al. 1999; Mohri et al. 2005). The first record of a large amount of Pacific bluefin tuna juveniles was from the survey during June 11–14, 1997, around the Kuroshio Current region north of the Okinawa main Island in the Nansei area by using a large mouth midwater trawl net (total length: 86 m, expected mouth opening: 30 x 30 m) (Fig. 2.5, Itoh et al. 1999). Based on molecular species identification of mtDNA haplotypes, 164 juveniles of Pacific bluefin tuna (19.5–46.9 mm FL, 35.7 mm mean FL) were collected (Chow et al. 2003) and found to inhabit
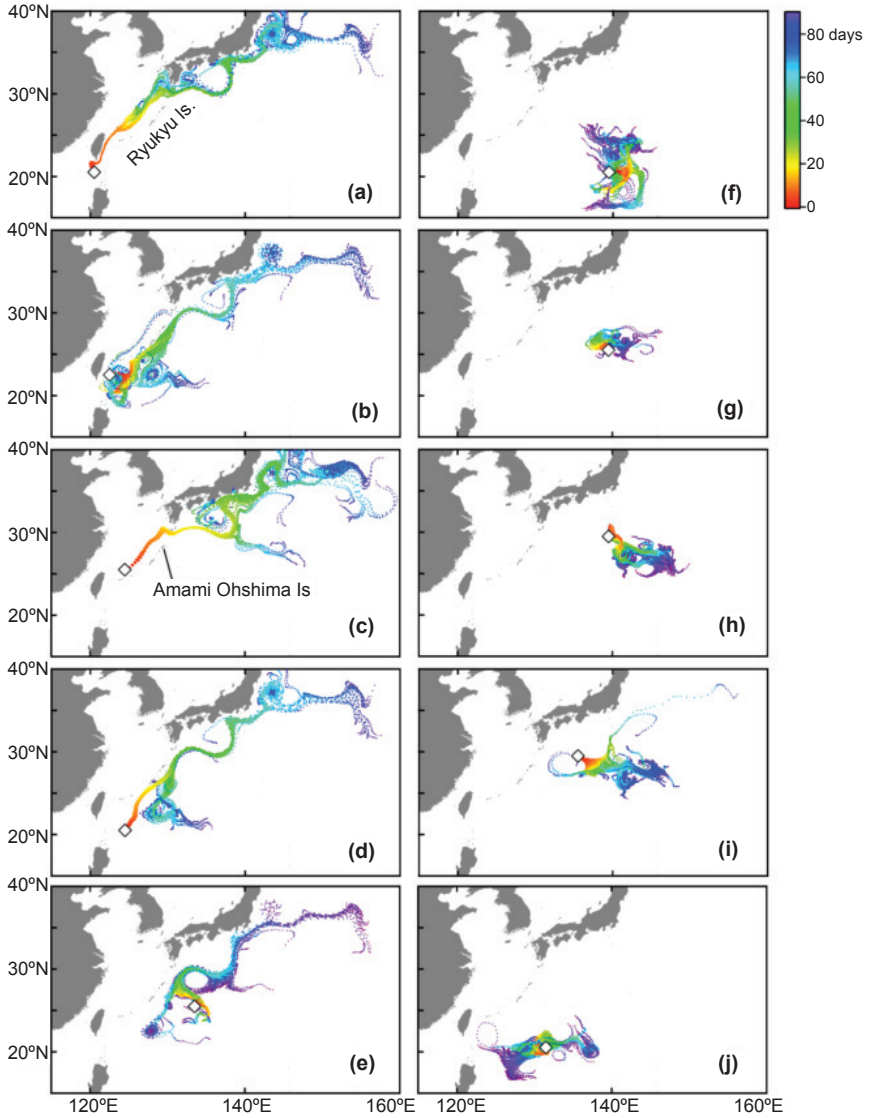
Figure 2.4. Representative particle trajectories from a release point (a) to the south (20.5°N, 120.5°E) and (b) east (22.5°N, 122.5°E) of Taiwan, (c) close to the Kuroshio (25.5°N, 125.5°E), (d) 20.5°N, 124.5°E and (e) 26.5°N, 129.5°E in waters on the eastern edge of the putative spawning area of bluefin tuna, and (f) 29.5°N, 130.5°E, (g) 25.5°N, 139.5°E, (h) 29.5°N, 139.5°E, (i) 29.5°N 135.5°E and (j) 20.5° N 131.5°E in waters outside of the putative spawning area. The diamond in each panel indicates the release point. The colors indicate the number of days after hatching (Kitagawa et al. 2010).
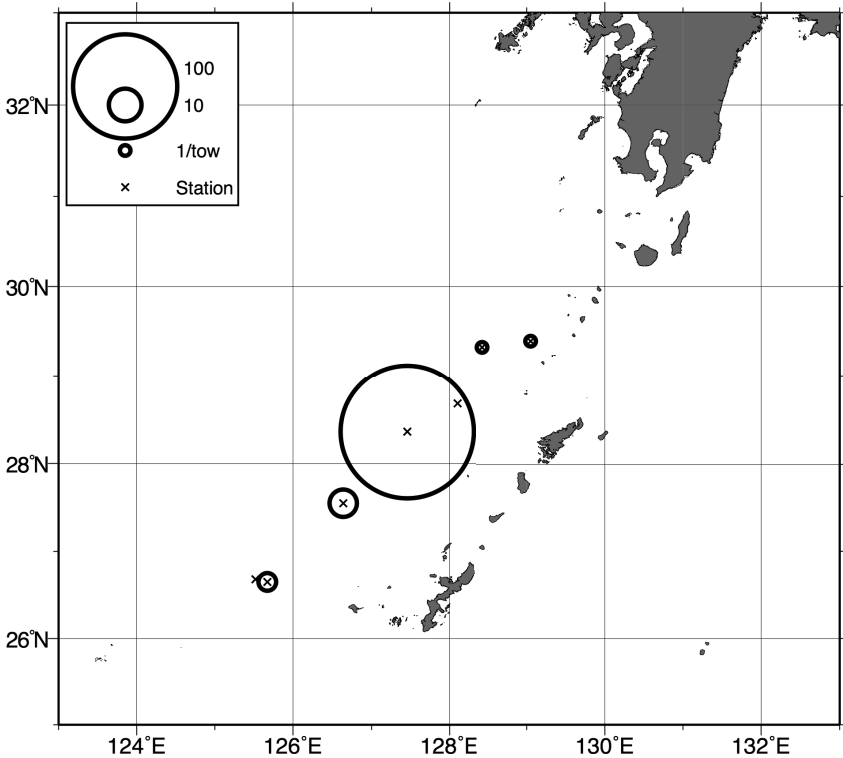
Figure 2.5. Horizontal distribution of juvenile Pacific bluefin tuna in the Nansei area in 1997. Modified from Itoh et al. (1999).

in waters within a range of 26.9–27.5°C of surface temperatures (Itoh et al. 1999). This finding was largely expected as the starting point to uncover early life history of the juvenile Pacific bluefin tuna including distribution, growth, feeding habitat, and recruitment process in the nursery area. Although a little success of such an amount of the juvenile catch has been achieved after that, trawl surveys have been continued around the the Nansei spawning ground to date.

Trawl surveys like those performed in the Nansei area were also carried out in the Sea of Japan. Mid-water trawls conducted from late August to late September in 1999 and 2004 succeeded to capture 100 and 86 individuals of Pacific bluefin tuna juveniles, respectively (Tanaka et al. 2007a). The juveniles appeared widely within the survey area in 1999 but restrictedly in the near-shore area in 2004 (Fig. 2.6), where mean ambient temperatures ranged from 23.4–25.9°C (mean from surface to 30 m deep), likely inhabiting at slightly lower temperatures than in the Nansei area. Since juveniles from the Sea of Japan (108–280 mm FL) were larger than those from the Nansei area (19.5–46.9 mm FL) sampled by Itoh et al. (1999), it was impractical to compare both results directly. Further surveys are expected to capture juveniles larger or smaller than 100 mm FL in the Nansei area or in the Sea of Japan, respectively. Accumulating knowledge from many more cases of Pacific bluefin juvenile catches would be crucial for illustrating distribution patterns and recruitment process of them.
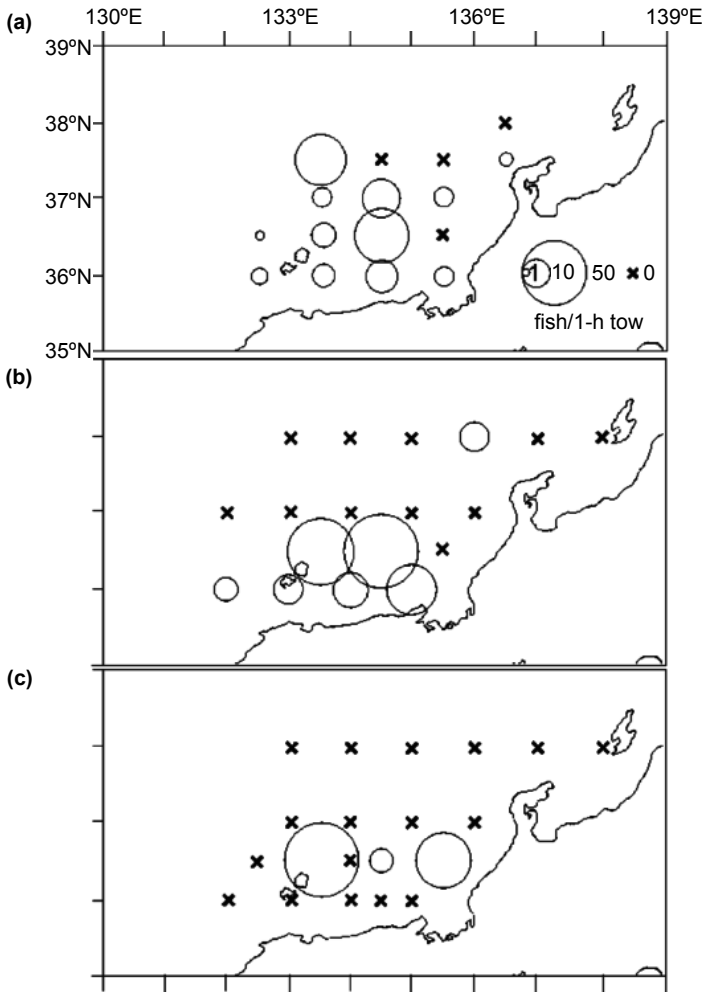
Figure 2.6. Horizontal distributions of juvenile Pacific bluefin tuna in the Sea of Japan from cruises in (a) 1999, (b) 2004 first cruise, and (c) 2004 second cruise (Tanaka et al. 2007b).

Eco-physiological Traits and Survival Strategy of Larval Pacific Bluefin Tuna

Growth and morphological development of larvae

Body length of newly hatched Pacific bluefin tuna larvae is 3 mm. Field-caught tuna larvae grow to 4.5 mm in Standard Length (SL) by seven days after hatching (DAH), 6 mm SL on 10 DAH and 7 mm SL on 14 DAH, estimated by otolith microstructure analysis (Tanaka et al. 2006). Morphological development of field-caught larvae has been reported in Tanaka et al. (2006). The shift from the preflexion to flexion phase is first noted at 4.0 mm SL on 7 DAH. Flexion larvae are observed up to 7.5 mm SL

on 13 DAH. Postflexion larvae are first noted at the 6.0 mm SL on 10 DAH and all captured larvae are in the postflexion phase at 7.5 mm SL on 14 DAH. However, fish in the late-larval and early juvenile stages have been rarely captured in the field.

On the other hand, information of the growth and morphological traits of hatchery-reared larvae and juveniles have been accumulated since Pacific bluefin tuna were reared under aquaculture conditions throughout their complete life cycle (Sawada et al. 2005). Hatchery-reared tuna larvae grow to 5 mm in Total Length (TL) by 10 DAH, 9 mm TL on 20 DAH and 30 mm SL on 30 DAH (Miyashita et al. 2001). Particularly, Pacific bluefin tuna larvae show very high growth rates after 20 DAH, which corresponds to the onset of piscivory (Tanaka et al. 2007b; Tanaka et al. 2014). Morphological developments of the hatchery-reared larvae are as follows (Fig. 2.7).
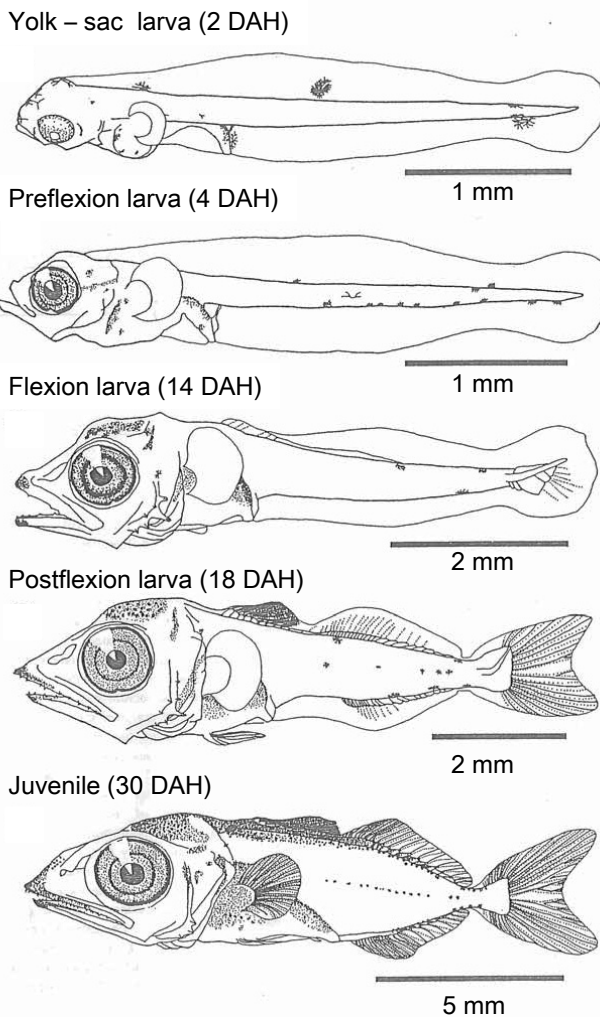
Figure 2.7. Morphological development of hatchery reared Pacific bluefin tuna (modified from Kaji et al. 1996).

The shift from the preflexion to flexion phase occurs at 5.0 mm SL on 10 DAH, from the flexion to postflexion phase at 7 mm SL on 14 DAH and from postflexion phase to juvenile at 16 mm SL on 23 DAH (Kaji et al. 1996; Tanaka et al. 2007b).

Precocious development of digestive system of Pacific bluefin tuna larvae

Generally, newly hatched larvae of most mass-spawning marine fish species are underdeveloped in terms with their digestive systems. The mouth and anus of the newly hatched larvae do not open and their digestive tract is a simple tube. Two transition periods in the process of development of the larval digestive system are observed. The former is the establishment of a primitive digestive system in the early larval stages, which enable to digest and absorb the exogenous nutrients. The latter is the establishment of an adult type digestive system which has a functional stomach with well-developed gastric glands and pyloric caeca occurring in the juvenile stage (Tanaka 1973).

The development of a digestive system of larval Pacific bluefin tuna is more precocious relative to other marine fishes. The gut opens from the mouth to anus on 2 DAH, differentiating the rudimentary stomach, intestine and rectum. The larvae at first feeding on 3 DAH have a primitive digestive system which enables the larva to feed on exogenous food although the gastric glands and blind sac is not differentiated (Kaji et al. 1996). By 12 DAH the stomach with a blind sac and gastric glands and the pyloric caecum become differentiated. At that time, an adult type digestive system is established (Kaji et al. 1996). Miyashita et al. (1998) also summarized the ontogenetic development of the digestive system. The pharyngeal teeth, mucous cells of oesophagus, blind sac and gastric gland differentiated in preflexion phase around 10 DAH. Pyloric caecum differentiated in the flexion phase on 15 DAH. Corresponding to the development of the digestive system, the activities of digestive enzymes increase. After the establishment of larval type digestive system, the activity of amylase rapidly increases. Thereafter, the activities of pepsin-like and trypsin-like enzymes quickly increase on 15 DAH immediately after the establishment of the adult type digestive system (Miyashita et al. 1998).

Thus, the larval and adult type digestive systems in the early life stages of Pacific bluefin tuna are established at first feeding on 3 DAH and on flexion phase on 12–15 DAH, respectively. The larval type digestive system of Pacific bluefin tuna is established in the typical periods of other marine fish species. However, the adult type digestive system establishes prior to the juvenile stage, which are earlier periods than other marine fishes. Pacific bluefin tuna shows rapid growth in the late larval to early juvenile stages after the onset of piscivory. The precocious development of the digestive system should be important characteristics for the rapid growth in the early life stages of Pacific bluefin tuna in relation to piscivory.

Ontogenetic changes in behavior in early life stages of Pacific bluefin tuna

The behavioral studies in early life stages of Pacific bluefin tuna have been conducted such as schooling and aggressive behavior using hatchery-reared fish in order to examine the problem of cannibalism that occurs in the mass culture process.

The swimming speed of tuna larvae is approximately 22.4 mm/s, and the speed does not change largely from the preflexion phase on 5 DAH to early juvenile on 21 DAH (Sabate et al. 2010). The swimming ability elevates with the increase of swimming speed after 25 DAH, reaching 500 mm/s on 55 DAH (Fukuda et al. 2010). The distance to the neighboring fish decreases, with the increase of the swimming speed. Swimming Separation Index (SSI) is often used as an index of schooling behavior (Nakayama et al. 2003). The value of SSI ranges between 0 and 2. When the value is 1.414, two fish show random swimming. The value decreases to 0 as the swimming of the two fish becomes aligned. The value of SSI of larval Pacific bluefin tuna is approximately 1.4 from 10 to 20 DAH (Sabate et al. 2010) (Fig. 2.8). Thereafter, the value of SSI is significantly less than 1.414 on 25 DAH after the transition to juvenile, which indicates the onset of schooling behavior. On 29 DAH, the value of SSI decreases to 0.5 and juvenile Pacific bluefin tuna shows clear schooling behavior. Pacific bluefin tuna develops schooling behavior later than other species such as anchovy *Engraulis mordax* (Hunter and Coyne 1982), Atlantic herring *Clupea harengus* (Gallego and Hearth 1994), yellowtail *Seriola quinqueradiata* (Sakakura and Tsukamoto 1999) and striped jack *Pseudocaranx dentex* (Masuda and Tsukamoto 1999), that develop schooling behavior in the late larval stage. The relatively late
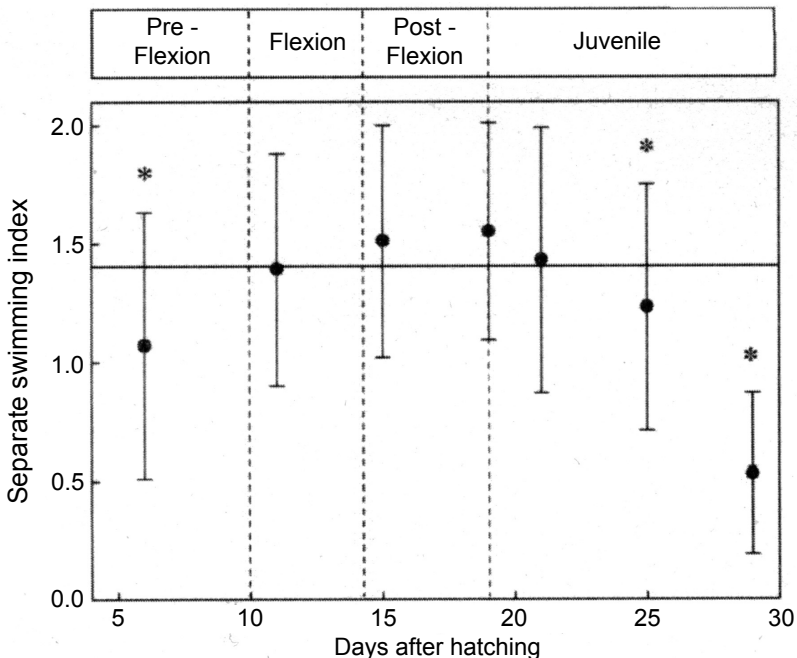
**Figure 2.8.** Changes in schooling behavior of Pacific bluefin tuna based on separation swimming index (SSI: Nakayama et al. 2003). Dots indicate the average SSI with standard deviation for every age group. Asterisks indicate significant differences between the mean and the expected SSI value for fish swimming at random direction and speed (t-test, $p < 0.05$) (modified from Sabate et al. 2010).

development of schooling behavior in Pacific bluefin tuna is presumed to reflect development of the central nerve system in the early juvenile stage, which is needed for schooling behavior (Sabate et al. 2010).

   In the process of mass culture, cannibalism of larvae and juveniles is frequently observed after the onset of piscivory, which is one of the causes of the low survival rate in hatcheries (Sawada et al. 2005). Aggressive behavior to neighboring individuals which induces the cannibalism first occurs in the postflexion phase (Fig. 2.9). Thereafter, the aggressive behavior is continuously observed after the juvenile stage (Sabate et al. 2010). The onset of aggressive behavior corresponds to the timing of the onset of piscivory.

   Pacific bluefin tuna larvae show piscivory with the development of aggressive behavior. The larvae which can feed on other fish larvae show high growth rates, consequently resulting high survival rates of the larvae in the field. The high growth rate can increase the swimming speed and rapidly develop the schooling behavior, which are advantageous to the foraging and escaping from predators. Thus, ontogenetic development of these behaviors should closely relate to the survival of larval and juvenile Pacific bluefin tuna in the field. Although these behavioral approaches are conducted using laboratory-reared fish, the results of behavioral studies will largely help the understanding of the mechanisms of transportation, survival process and estimation of the recruitment abundance in the early life stages of Pacific bluefin tuna in northwestern Pacific Ocean.
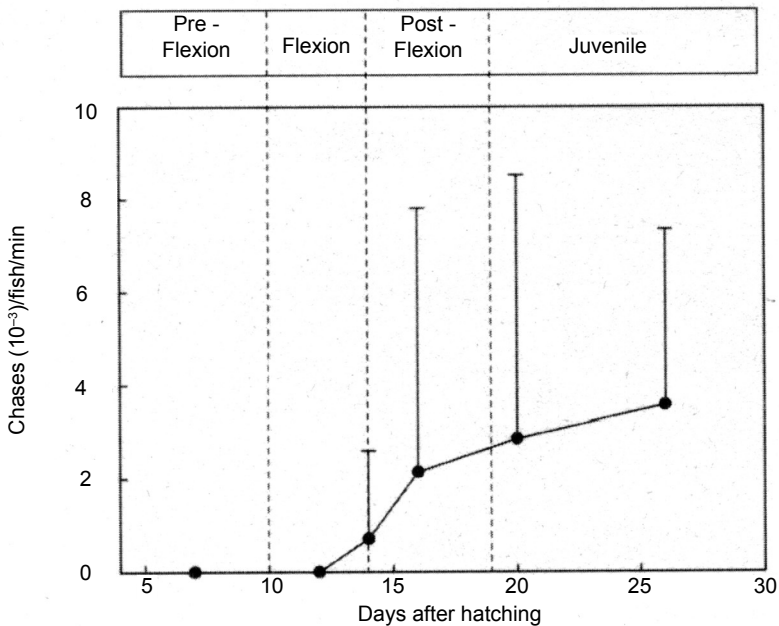
Figure 2.9. Mean number of chases per minute with standard deviation as an estimate of aggressive behavior in Pacific bluefin tuna (modified from Sabate et al. 2010).

Feeding habits

Pacific bluefin tuna larvae are visual day feeders and they do not feed at nighttime (Uotani et al. 1990). Uotani et al. (1990) examined the gut contents of 1939 Pacific bluefin tuna larvae (2.28–14.60 mm BL) collected at the main spawning ground for the present species in the northwestern Pacific Ocean. The main prey item of the larvae is copepods, accounting for 97.0% in number (Table 2.1). The larvae smaller than 5 mm BL mainly preyed on copepod nauplii smaller than 0.3 mm, while copepodites of genus *Corycaeus* were the main component for the larvae larger than 5 mm BL. Thus, a shift in feeding habit is observed at 5 mm BL, from small zooplankton (copepod nauplii) to larger zooplankton (copepods of *Corycaeus* spp.). The relative growth of the head length, mouth size and body depth of larval Pacific bluefin tuna changes at approximately 5 mm BL (Uotani et al. 1990; Miyashita et al. 2001). Furthermore, the pharyngeal teeth differentiate at this size (Miyashita et al. 1998). The shift in feeding habit at 5 mm BL is observed corresponding to these morphological changes, which could be due to the improvement in ability to catch and digest the prey.

Although Pacific bluefin tuna larvae tend to prey on larger zooplankton with fish growth, prey fish larvae were not found in their guts in the report of Uotani et al. (1990). On the other hand, laboratory-reared larvae show piscivory from sizes larger than 8 mm SL (Tanaka et al. 2014). However, the larvae ranging 10 to 20 mm SL have been rarely captured in the field and the ecological traits in relation to piscivory of field-captured larvae are still unknown. Since the piscivory could play an important role for the early growth, survival and consequent recruitment success of Pacific bluefin tuna, it is expected to elucidate the feeding habits in the late larval to early juvenile stages.

Table 2.1. Number and frequency of occurrence of forageorganisms found in the guts from 1939 larvae (Uotani et al.1990).

Food item	Number of organisms	Frequency of occurrence (%)
COPEPODA	1885	97
Paracalanus	62	3.2
Clausocalanus	217	11.2
Corycaeus	722	37.1
Temora	1	0.1
Oncaea	1	0.1
Copepoda nauplii	667	34.3
Copepoda eggs	57	2.9
unidentified Copepoda	158	8.1
OTHERS	61	3.3
Evadne	58	3
Mysidacea	1	0.1
Macrurura nauplius	1	0.1
Fish egg	1	0.1
	1946	100.3

Survival mechanism in relation to growth and nutritional condition

Pacific bluefin tuna produce a huge number of small pelagic eggs. The newly hatched larvae are very vulnerable. These small larvae experience various environmental conditions through their ontogeny. Then they show heavy mortality due to the dispersion, predation, starvation and disease and so on, and a very few survived fish can recruit to the stock. The survival mechanisms in early life stages of mass-spawning fishes are concerned with the population dynamics. The elucidation of the survival mechanisms in early life stages is indispensable for optimal stock management. Here, we describe the survival mechanisms in the early life stages of Pacific bluefin tuna in the northwestern Pacific Ocean in relation to their growth and nutritional condition.

The otolith microstructure has daily increments which enable estimation of the daily age of fish by enumeration of the increments number. The increment width is generally proportional to the somatic growth (Campana and Neilson 1985). Because the growth history of each individual is recorded in the otolith (Degens et al. 1969; Dunkelberger et al. 1980; Watanabe et al. 1982; Mugiya 1987), growth histories can be back-calculated.

The otolith microstructure analysis of tuna larvae collected around the Ryukyu Islands in the northwestern Pacific Ocean revealed that marked growth and developmental variations of the larvae occur even at the same age (Tanaka et al. 2006). Juvenile tuna ranging from 15 to 30 cm TL are caught in Japan by troll fisheries in the coastal areas off Kochi and Nagasaki prefectures in July and August. These juveniles are considered to be survivors of the larval cohorts distributed around the Ryukyu Islands. In Tanaka et al. (2006), the growth histories of juveniles in their larval periods were compared to those of larvae at each age ranging from 6 to 13 DAH, estimated by otolith microstructure analysis. Figure 2.10 shows frequency distributions of logarithm otolith radius (ln OR) of larvae in the preflexion, flexion and postflexion phases and back-calculated for juvenile tuna at 10, 11, 12 and 13 DAH. Since SL was positively correlated with the ln OR during the larval stage, ln OR were used for the index of SL at each age. Arrows indicate smallest value of ln OR in juveniles and represent the smallest possible size of larvae for successful recruitment to juvenile stage, indicating that the larvae with smaller otolith than that value did not survive to juvenile stage. A large part of preflexion, and flexion larvae had smaller otoliths than those of the juveniles. On the other hand, the otolith radius of postflexion larvae was comparable to the juveniles. The results showed that surviving juveniles originated from the larvae with high growth and developmental rate at catch among the larvae with various growth and developmental rate distributed around the Ryukyu Islands.

Growth of larvae and juveniles is one of the most important factors for their survival. In several fish species, larvae with lower growth rates have been reported to have a higher mortality (Hovenkamp 1992; Meekan and Fortier 1996; Searcy and Sponaugle 2001; Takasuka et al. 2003; Takahashi and Watanabe 2004), because bigger larvae have a higher tolerance to starvation and a greater ability to escape from predators than smaller larvae (Anderson 1988; Miller et al. 1988; Bailey and Houde 1989). In Japanese anchovy, growth and developmental rate-dependent mortality occurred at 50 to 60 days. Growth selective mortality occurred at 41 to 80 days in Atlantic cod *Gadus morhua* (Meekan and Fortier 1996). In the case of Pacific bluefin