New Discoveries in the Olfactory Capability of Sea Turtles

Richard G. Vogt, Michelle Vieyra and Daniel Anderson

This is a preliminary report of results obtained in a continuing study of the sensory capabilities of sea turtles, conducted in collaboration with Dr. Richard Brill of the National Marine Fisheries Service. Funded through the Pelagic Fisheries Research Program, these experiments began in July 2001, and were performed in the Department of Biological Sciences at the University of South Carolina, Columbia.

How important is the sense of smell to a sea turtle? And if it is important, can we make use of smell to more effectively manage turtle populations— for example, to reduce the numbers taking baited fishhooks? All organisms interpret and interact with the world through sensory systems, without which they could find no mates, avoid no predators, or locate no food.

The ability to detect chemicals, which we think of as smell and taste, is perhaps the oldest sense— present in bacteria since the beginning of life and in every organism since. Smell and taste are less different in what they detect in how the detection is accomplished. Humans smell and taste very different kinds of chemicals, but fish smell and taste very similar chemicals. One consistent difference between smell and taste is that each sends information to a different part of the brain. Another difference is the way in which smell and taste are used. In general, smell works from a distance, while taste works close up. For example, animals make food decisions in a hierarchical fashion: seeing and/or smelling something from a distance arouses interest, after which biting and tasting provides information about nutritional or toxic content. Making fish bait unattractive to sea turtles' noses might prevent them from contacting fishhooks altogether.

How Are Odors Detected?

Odors are detected by proteins called odor receptors (ORs) embedded in the membranes of olfactory cilia, which protrude from olfactory sensory neurons (see Figure 1). The interactions between odor molecules and ORs are the all-important initial recognition step in odor detection. Supposedly, humans can discern 10,000 different odors, accomplished by having about 350 different ORs, each one individually expressed in about 1000 sensory neurons. Any given odor activates a small but unique combination of different ORs and neurons. Mice and rats accomplish this detection with about 1000 distinct ORs, and fish with about 100 ORs. In the evolution of vertebrates (see Figure 2) there has been an increase in the number of OR genes (fish have 100 ORs, mammals have 1000), as well as an occasional decrease in the numbers of genes that are actually functional.

ORs and Olfactory Utility

A great deal is known about vertebrate ORs and OR genes; in particular, it is known that the structure of OR genes is very similar in vertebrates from fish to humans. We believe we can make use of this fact to assess in a non-invasive manner certain aspects of the sense of smell in threatened and endangered species. Our objective is to determine the value of smell-based strategies now being developed to manage these species.

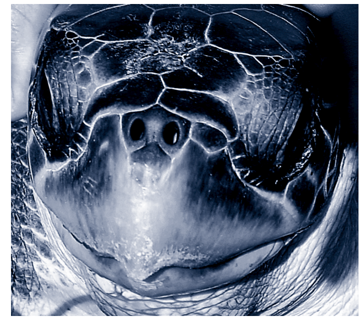

Figure 1. Face of an Olive Ridley Sea Turtle showing prominent nasal openings to olfactory tissue. Turtle was rescued from longline fishhook off Costa Rica in November 2001. Photo courtesy of Richard Brill and Yonat Swimmer, NMFS, Honolulu.

Scientists have isolated and characterized the DNA that encodes the proteins for many ORs in the coelacanth, 1 bird species, 2 frog and 1 salamander species, numerous mammal species, and several fish species (Figure 3). However, nothing is known of the ORs in turtles and reptiles. When vertebrate ORs are compared, very similar amino acid sequences are seen in specific regions of all, providing both hallmarks that help identify the sequences as ORs, and tools that are useful for cloning ORs from new species such as

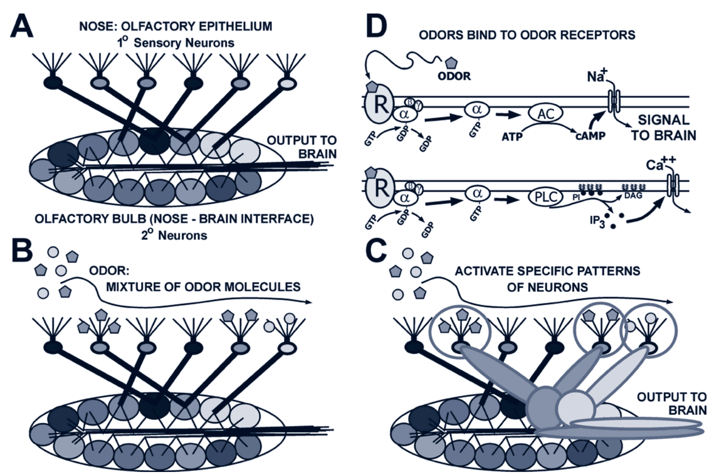

Figure 2. In all vertebrates, odors are detected by primary sensory neurons in an olfactory epithelium located in the nose (A). The primary neurons connect to secondary neurons in the olfactory bulb in spherical structures called glomerulae; the secondary neurons send signals further into the brain. Odors are usually mixtures of different chemicals; each chemical is detected by a unique set of sensory neurons, which in turn connect to and activate a unique pattern of glomerulae and secondary neurons, sending a specific signal to the brain (B, C). Odor molecules bind to and activate Odor Receptor proteins (ORs) located in the membranes of the sensory neurons. The activated ORs then activate a biochemical pathway that generates an electrical signal (D). Interaction between odor molecules and ORs is the initial recognition step in odor detection. For more information, read the review by Hildebrand and Shepherd, 1997.

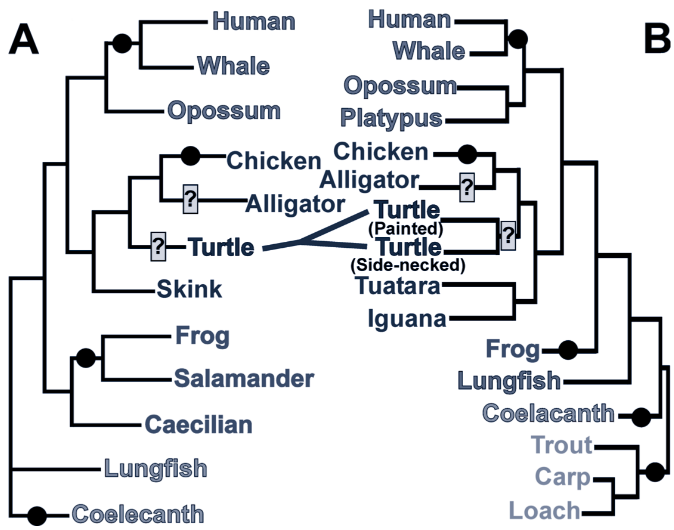

Figure 3. Current views of the evolutionary relationships of these animals, indicating the relatively close relationship between turtles, birds and reptiles (Zardoya & Meyer, 1998 (B), 2000 (A)). Vertebrates include about 45,000 of the 1.5 million known species of plants, animals, etc. on the planet. Fish, amphibians, birds and reptiles (including turtles), and mammals appeared about 450, 365, 300 and 220 million years ago respectively. Odor receptors are known from those lineages marked with filled circles. Nothing is known of odor receptors from turtles or reptiles ("?").

turtles and reptiles. We arranged to have small pieces of DNA made that match these conserved regions of OR DNA, and used these pieces as primers in a technique called Polymerase Chain Reaction to amplify and clone OR genes; the genes come from genomic DNA isolated from the blood of Green, Leatherback and Loggerhead Sea Turtles, and the American Alligator.

This was entirely non-invasive— the blood samples were already in storage as part of ongoing population genetics studies used for the management of each species. A drop of one of these samples (about 0.2 ml) was all that was needed to identify many OR genes. While still preliminary (our study began July 2001), we have identified nearly 20 OR genes from both Leatherback and Loggerhead Sea Turtles, about 10 OR genes from Green Sea Turtles, and 6 OR genes from alligators; these numbers are increasing as work continues. What is this new information telling us, and how can we use it?

The Nature of Sea Turtle ORs

Sea turtles have ORs that are both similar to and distinct from the ORs of other vertebrates (see Figure 4). Initially, we identified sea turtle ORs by comparing their sequences with those of other vertebrate species. Sea turtle ORs are more similar to those of

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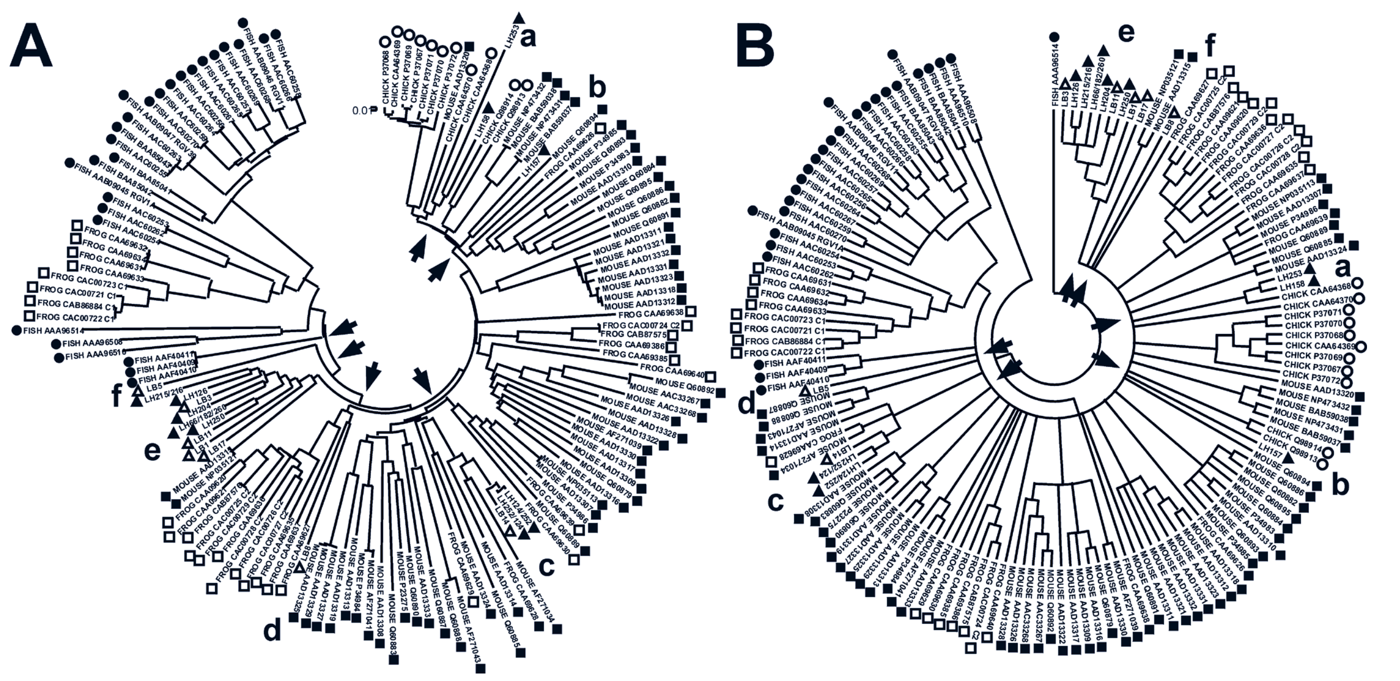

Figure 4. Comparison of Loggerhead and Leatherback OR sequences with those of other vertebrates. Amino acid sequences of proteins were first aligned; subjected to a computer program (Clustal W; Thompson et al., 1997) that makes a large table in which each protein occupies one row organized so that equivalent amino acids are in the same columns. The table is then subjected to a second computer program (MEGA2; Kumar et al., 2001), which tries to identify which sequences are most similar. The result is a dendrogram in which supposedly related sequences are found in the same branch. In this figure, ORs of Loggerhead (LH, filled triangles) and Leatherback (LB, open triangles) Sea Turtles are compared with all known OR sequences of zebrafish (FISH, filled circles), xenopus (FROG, open squares), chicken (CHICK, open circles) and mouse (MOUSE, filled squares. Arrows and letters (a-f) help locate turtle sequences. In A, branch lengths are proportional to the amino acid difference in the sequences. In B, branches are only shown for those relationships considered statistically meaningful ("50% bootstrap, 10,000 replicates"), branches are not proportional. In this analysis the dendrograms were made by a method called "neighbor joining."

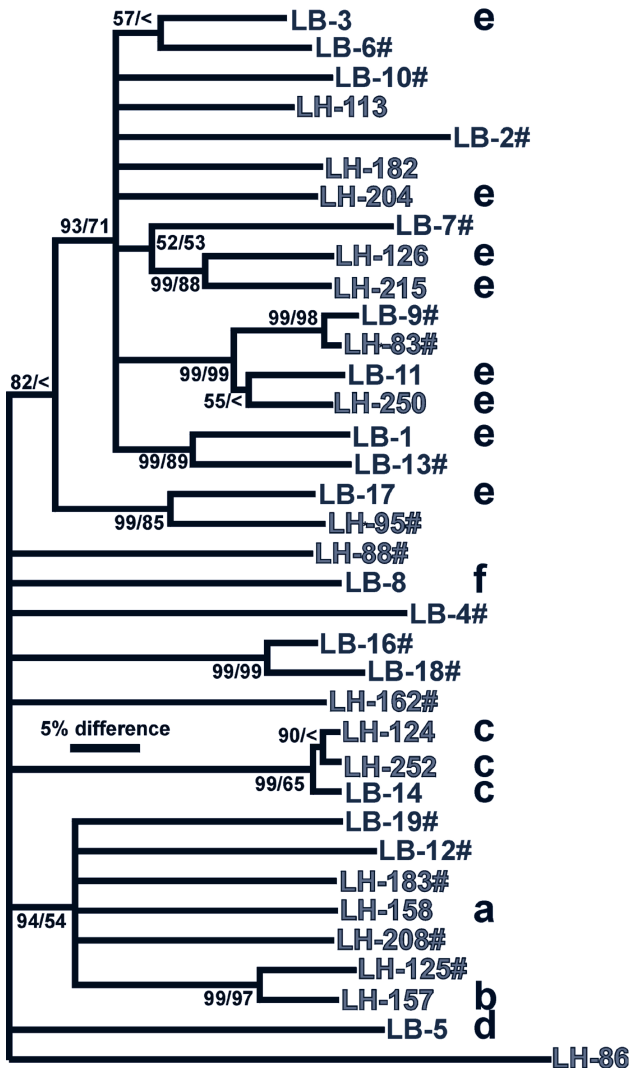

Figure 5. Comparison of Loggerhead and Leatherback OR sequences. Nucleic acid sequences (genes) were compared as described in Figure 4. Sequence names marked with "#" contain defects ("internal stop codons") suggesting that they are not expressed (i.e. non-functional). Numbers indicate statistical support ("bootstrap value") for branches from different analyses; the higher the number the stronger the support (only branches >50% are shown). Letters (a-f) identify the same sequences as in Figure 4. A dendrogram is a neighbor-joining tree, in which branch lengths are proportional to the sequence difference.

mammals, birds and frogs than they are to fish, which is consistent with the evolution of these animal groups (Figure 3). But sea turtle ORs also are distinct enough to fall into turtle-specific groupings. Important for our subsequent analyses, the sea turtle ORs fall into several groupings, distributing with a range of OR types from other vertebrates. Assuming that one can relate protein sequence to protein function, this distribution suggests that we have identified OR genes that have a broad functional repertoire.

Our analysis indicates that at least half of the OR genes in the sea turtle genome do not function at all (see Figure 5). When our cells make proteins, the gene DNA is first copied into mRNA, and the mRNA is then translated by ribosomes that assemble the protein. The mRNA contains a stop signal ("stop codon") that tells the ribosome when the end has been reached. More than half of the sea turtle OR sequences we have analyzed have incorrectly placed stop codons, indicating that if these genes were expressed, their products (proteins) would not work. Non-functional OR genes are not uncommon, but tend to occur in specific vertebrate groups, and are thought to represent a secondary loss of genes rather than an incomplete initial gain of genes. In primates, including humans, only about 30% of OR genes are functional (Rouquier et al., 2000; Zozulaya et al., 2001). All identified OR genes in dolphins are non-functional, consistent with their reduced olfactory bulb and suggestions that dolphins lost their sense of smell when their ancestors became aquatic (Freitag et al., 1998). On the other hand, most mice OR genes are functional. A reduced population of OR genes in sea turtles is certainly curious, and may reflect an alteration in their olfactory capability that accompanied a transition from terrestrial life to marine life.

Number of OR Genes in Sea Turtles

We are nearing the end of our initial phase of identifying OR genes in our chosen species. We can now estimate the number of OR genes, and compare this with the numbers of expressed vs. non-expressed OR genes (see Figure 6A). This will provide an estimate of the olfactory potential of these animals and of the diversity of odor molecules they might be capable of detecting. These experiments will involve southern blot analyses comparing the sea turtle OR hybridization complexity with that in zebrafish, which have about 100 OR genes, and in mice, which have about 1000 OR genes. This may provide important additional insights regarding when exactly the expansion of OR genes occurred in the evolutionary history of vertebrates.

Significance of Olfaction in Sea Turtles

This is the ultimate question. Multiple versions of the same gene exist in a population of animals, providing some of the bases for individual variation; these gene versions are called "alleles." Geneticists identify distinct populations of members of a given species by comparing the ratios of different alleles of specific genes; distinct ratios imply a low level of interbreeding and gene mixing between two populations. We can use this technique to estimate the importance of OR genes in behavior, by comparing the sequences of alleles of several specific OR genes from many individuals of a population (see Figure 6B).

High allelic diversity would result from a lack of selective (behavioral) pressure favoring any one gene form, and would indicate that olfaction may not be that important. But if allelic diversity is low, with one form predominating over the others, we may hypothesize that there is significant selective pressure favoring one form over the others, and that olfaction is important in the animal's behavior. Many individual blood samples already exist from

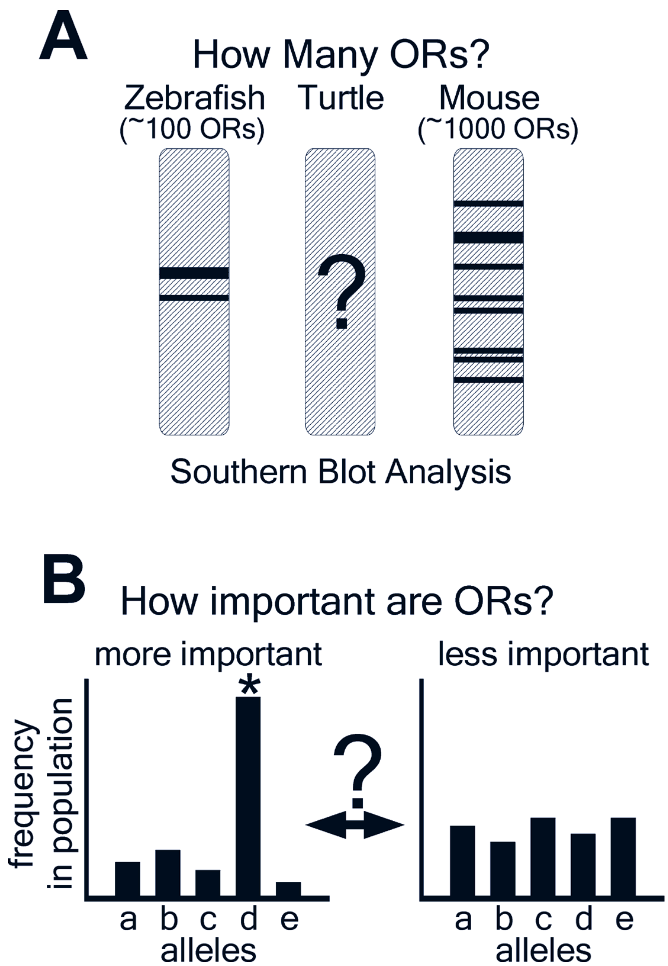

Figure 6A. The number of OR genes in an animal can be estimated by performing Southern Blot Analysis. Genomic DNA (i.e. chromosomes) is isolated and enzymatically degraded in a controlled manner, after which the resulting fragments are separated by electrophoresis. A DNA probe (e.g. a turtle OR gene) is used to identify similar sequences. The number of bands reflects the number of genes.

Figure 6B. The importance of ORs in an animal's life can be estimated using population genetics. All genes are present in a population of animals in multiple forms, called alleles (one of the main reasons we are all different). Comparing the sequences of a specific OR gene from many individuals identifies the ratio of different alleles in the population. An unusually high proportion of one allele suggests that allele is better for the animal in some way and that the gene is playing an important role in the life of the animal.

populations of Leatherback and Loggerhead Sea Turtles and are available for such analysis.

The Value of Olfactory Based Management

Management of endangered or threatened species such as sea turtles requires a gentle hand. We believe that the importance of chemosensory information can be assessed non-invasively through molecular analysis of pre-existing tissue samples. The approach outlined can be applied to any animal species. In this study, our molecular experiments are being performed in parallel with careful behavioral studies by Drs. Yonat Swimmer and Richard Brill (NMFS, Honolulu) (see Figure 7). Together, these projects will clarify how Sea Turtles' chemo-sensory systems can be exploited to maintain a more effective distance between turtles and longline fishing gear.

Figure 7. Testing the chemosensory response of Green Sea Turtles: in this case, the turtle is attracted to chopped squid inside the black plastic container that obscures visual characteristics of the squid. The experiments were conducted as a component of this project by Yonat Swimmer and Richard Brill, NMFS, Honolulu, December 2001.

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MHLC7— Evaluation and Comment

Following is the fourth in a series of commentaries on the Multilateral High-level Conference on the Conservation and Management of Highly Migratory Fish Stocks in the Western and Central Pacific (MHLC). The goal of this series is to share the evaluations of scientists and other parties who are well informed about the MHLC, its Convention, Preparatory Conferences and evolving Commission, in the hope that continued frank discussion can contribute to the most effective and mutually agreeable implementation of the Convention.

We have accepted the assessments of persons recommended as knowledgeable about MHLC7 and are seeking additional comment. Our principal criteria are that contributors are familiar with the proceedings, the science and the proposed management schemes, and are willing to answer a common set of questions, with an opportunity for open comment.

Comments, questions, and requests for inclusion as a contributor may be addressed to Editor, PFRP News, 1000 Pope Road, MSB 313, Honolulu, Hawai'i 96822, or e-mailed to andercox@hawaii.rr.com, or jsibert@soest.hawaii.edu.

Robin Allen

The MHLC was conducted to negotiate an international fisheries management arrangement for the stocks of highly-migratory fish in the Western and Central Pacific Ocean (WCPO). While the Conference succeeded in the sense of adopting a Convention, there is still much to be done before a management arrangement that is supported by all participants in the MHLC is achieved. There will not be an accepted framework for conservation and management until the major fishing states and the coastal states agree to cooperate within the framework of the Convention. Until then, the objective of the Conference will not be achieved.

While these issues are being resolved, institutional arrangements for data collection and research apparently are being set back. In the aftermath of the Convention, research cooperation seems to have diminished, and some positions for alternative arrangements seem to be developing. However, that is not to say that the objectives will not be achieved eventually. It is possible that sufficient differences in the positions that remained after the MHLC can be resolved with further patient negotiation to bring about the sought-after cooperation.

Against that background, I offer my thoughts on the facilities for data collection and research envisaged by the Convention. The Convention establishes a Scientific Committee composed of representatives of members of the Commission[1], and contemplates that the Commission would engage external scientific services. This takes a middle ground between conventions such as that of the Inter-American Tropical Tuna Commission (IATTC), which provides for a permanent scientific staff, and others that establish scientific committees but not permanent scientific staffs reporting to those commissions. The contrast between the two extremes is not as sharp as it was formerly, as other commissions have strengthened their own technical staffs, and IATTC stock assessments and other scientific advice are now reviewed by a scientific working group composed of representatives of member and interested countries. Apparently these developments acknowledge the utility of both a scientific staff and a scientific committee.

Contracting Scientific Services

The scientific services contemplated by the Convention could be implemented in various ways that would have different implications for data collection and research. The following comments refer to the outcome that I see as being most effective: that is, a long-term relationship with a group who could act in a manner similar to that of a permanent staff. At a minimum, the duties of such a group would include stewardship of the Commission's data, and provision of stock assessment and scientific advice.

This would require a larger Commission budget than an arrangement with more modest scientific services. However, it does not necessarily imply greater costs for members of the Commission, who have the common obligation of providing data and scientific information to help manage the fishery. Without a central service, the members would have to contribute data management and stock assessment individually, through their own internal resources. In fact, a dedicated team with an overview of the entire fishery would be able to meet those needs more efficiently; such a team would be able to deploy resources effectively and avoid unnecessary duplication of effort.

In addition to efficiency, a contracted scientific service has other advantages for most members of the future Commission. An organization providing contracted scientific services would be equally responsible to all members. This would assure states that contribute detailed catch and effort data that their data would be used for scientific purposes without disclosing confidential information to others. Further, the likely members of the Commission range from states with well-developed scientific facilities to those with minimal facilities. While scientists always aim to provide objective results, experience in fisheries commissions indicates

[1]The Commission for the Conservation and Management of Highly Migratory Fish Stocks in the Western and Central Pacific Ocean.

that there is a tendency for the agendas and perspectives of scientific delegations to reflect national views. Also, the views of those with the best-developed scientific facilities are likely to dominate the Scientific Committee. Contracted scientific services can balance this asymmetry of advice, and provide all members with an independent source of scientific expertise.

A contracted scientific service could also provide support for data collection and biological research, although the arguments for doing this are less compelling than those for data management and stock assessment.

How Big a Budget?

The question that is yet to be answered is the budget needed to provide adequate scientific services. The IATTC (because the author is familiar with this organization's budget) could be used as a guide for this. The costs of contracted scientific services are likely to be similar to those of a permanent staff. The IATTC currently has a research budget of about US$3 million (excluding dolphin-related work), which provides for data collection and management, biological research, and stock assessment. The stock assessment focuses on yellowfin, bigeye and skipjack tunas, with less intensive research directed toward bluefin tuna and billfish.

The new Commission similarly will need to have access to assessments of all the stocks of highly-migratory species in the convention area, especially those that are fully exploited or nearly so. Due to the significantly greater size of the fisheries in the WCPO relative to those in the Eastern Pacific, the data management responsibilities are likely to be more complex and costly than those of the IATTC. Even if contracted scientific services do not include data collection and biological research, funding on a scale similar to that of the IATTC would be necessary.

The Convention spells out the obligations of members to contribute necessary data, and establishes a regional observer program and a vessel-monitoring system. If these facilities are implemented, the new Commission will start with an unrivalled access to data, which will augur well for the scientific understanding of the resources and their fisheries. However, the key to cooperation and data provision is, of course, not the language of the Convention, but the willingness of participants in the fishery to trust one another and work together for the common good. At the moment the prospects for that seem bleak.

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Robin Allen is Director of the Inter-American Tropical Tuna Commission. He first became involved in tuna research while at the University of British Columbia, when his thesis included modeling the Eastern Pacific Yellowfin Tuna stock. Subsequently he has worked in fisheries research and management for the New Zealand Government and the IATTC.

Compendium—Fisheries Research in Brief

SCTB Returning to Honolulu

The Fifteenth Meeting of the Standing Committee on Tuna and Billfish (SCTB) will be hosted by the PFRP in Honolulu July 18–27, 2002—and is expected to attract special attention because the nascent Western and Central Pacific Fisheries Commission (of the MHLC) will be indirectly seeking SCTB advice on the status of major tuna stocks in the region.

The MHLC (Multilateral High-level Conference for the Conservation and Management of Highly Migratory Fish Stocks in Western and Central Pacific) concluded in Honolulu in September 2000 with its namesake treaty. This treaty created a Preparatory Conference (PrepCon) to establish the Commission, which is intended to help implement the provisions of the treaty.

The second session of the PrepCon was held late in February in Papua New Guinea. Among its conclusions was a decision to establish a "Scientific Coordinating Group" (SCG) to receive information on the status of skipjack, yellowfin, bigeye and southern albacore tuna, and provide interim management advice to the PrepCon. The first meeting of the SCG will follow SCTB15 on June 29 & 30 at the East-West Center in Honolulu.

Further details and a provisional agenda for SCTB15 can be found on the PFRP website, and an article descriptive of SCTB14 can be found in the October–December 2001 issue of the PFRP newsletter (vol. 6, no. 4). A full report of PrepCon 2 will appear in the next PFRP newsletter.

MHLC Grows in Membership/Funding

The Chairman of the Preparatory Conference for the Multilateral High-level Conference on the Conservation and Management of Highly Migratory Fish Stocks in the Western and Central Pacific (MHLC) announced on January 24 that the Republic of Korea donated US$50,000 to support the Government of Papua New Guinea as host of the second session of the PrepCon. The donation represented Korea's contribution to the PrepCon Organizational Fund, and was made on the same day that Papua New Guinea allocated Kina 100,000 as its contribution to the Fund.

In addition, the PrepCon Secretariat announced that it had received a written petition from the State Committee for Fisheries of the Russian Federation requesting an invitation to participate in PrepCon deliberations. Vyacheslav Volokh, the Federation's Committee Deputy, noted in the petition that the Federation is a coastal country within the area of the

(continued on page 12)

Compendium (continued from page 11)

Convention, that it is able to fish for tuna in the area, and that it therefore is lawfully entitled to participate in the process of determining conservation and management measures as provided in UNCLOS–1982, and in the FAO Code of Conduct for Responsible Fisheries–1995. The Secretariat resolved to consider the petition during PrepCon 2, recently concluded in Papua New Guinea.

Fishing Scars Atlantic Reefs

New research suggests that fishing trawlers are smashing ancient coral reefs in cold, deep waters of the Northeast Atlantic—and marine scientists are calling for urgent protection of the unique and poorly understood reefs. "The best way to protect these areas is a blanket ban on bottom trawling," says Jason Hall-Spencer of the University Marine Biological Station. Coral experts hope a forthcoming overhaul of European Union fishing rules will provide an ideal opportunity to impose such a ban. For details, see: Hall-Spencer, J., Allain, V. and Fossa, J. H. "Trawling damage to Northeast Atlantic ancient coral reefs." Proceedings of the Royal Academy of Sciences B, online publication DOI: 10.1098/rspb.2001.1910 (2002).

(from Nature, *Science Update, 26 February 2002)*

Reserves Bolster Fish Stocks

Researchers have concluded that banning fishing in some areas does indeed boost catches in others. Fisheries in the Caribbean and Florida have become more productive since marine reserves were established, despite fishermen having fewer areas to fish in. The idea that such reserves help fishing has been controversial. "There hasn't been good evidence that reserves will benefit surrounding fisheries," says Callum Roberts, a conservation researcher at the University of York, UK—but he thinks his latest research provides that evidence. For details, see Roberts, C. M., Bohnsack, J. A., Gell, F., Hawkins, J. P. and Goodridge, R. "Effects of marine reserves on adjacent fisheries." *Science*, 294, 1920–1923, (2001).

(from Nature, *Science Update, 30 November 2001)*

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