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ARTICLE

Recommendations and Action Plans to Improve Ex Situ Nutrition and Health of Marine Teleosts

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Abstract
The International Workshop for Ex-Situ Marine Teleost Nutrition and Health, hosted by Disney's Animals, Science and Environment in conjunction with the Comparative Nutrition Society, brought together over 50 animal experts and scientists representing 20 institutions to review current science and identify challenges of marine teleost nutrition and health. Invited speakers presented critical information and current research topics for areas of emphasis and expertise. Subject matter experts identified knowledge gaps and primary areas of focus to guide the scientific community's research efforts to improve the care of ex situ marine teleosts. The clinical medicine working group highlighted standardized approaches to ante- and postmortem sample collection, diet biosecurity and supplementation, advanced diagnostic methods, and expanded training in fish nutrition. Nutrition identified the creation of a husbandry and feeding management manual, comprehensive feeding program review and design, and specialty feeder/life stage nutrition as areas of focus, while animal husbandry focused on body condition scoring, feed delivery techniques, and behavioral husbandry topics. The physiology and chemistry and water quality working groups discussed components of the aquatic environment and their effects on fish health, including organic matter constituents, microbial diversity, disinfection, and managing microbiota. Finally, we reviewed how epidemiological approaches and considerations can improve our evaluation of aquarium teleost nutrition and health. The goals outlined by each working group and supporting literature discussion are detailed in this communication and represent our goals for the next 3 to 5 years, with the ultimate objective of the workshop being the production of a husbandry manual for marine teleost nutrition and health. Any scientists who feel that their experience, research, or interests align with these goals are invited to participate by contacting the authors.

In January 2018, Disney's Animals, Science and Environment partnered with the Comparative Nutrition Society to present the International Workshop for Ex-Situ Marine Teleost Nutrition and Health. Over 50 experts in nutrition, clinical medicine and pathology, animal husbandry, physiology, water chemistry, toxicology, and epidemiology, representing 20 institutions, discussed the current science, research, and knowledge base for marine teleost nutrition and health. The purpose of the workshop was for experts to identify knowledge gaps and develop action plan recommendations that could guide the scientific community's research priorities to improve the care of ex situ marine teleosts. In the ex situ management of teleost species, the challenges can be as numerous and varied as the number of teleost species themselves found worldwide. Much of our current knowledge is limited to aquaculture species and institution-specific best practices. In aquaria, we often design nutrition plans to offer a broadly balanced diet to many unique species, often originating from many habitats throughout the world's oceans; various species often represent unique dietary strategies and/or feeding adaptations within their natural ecosystems. Our nutrition, husbandry, and medical care teams seek to provide the highest quality animal welfare possible. Each aspect of animal care is interdependent and often necessitates collaboration to maximize health outcomes. It was under this spirit of connection and cooperation that we chose to bring together this diverse group of specialists. By fostering connections and working together as a cohesive, multidisciplinary group of scientists across specialties, we sought to maximize research outcomes and, in turn, maximize animal welfare outcomes for all ex situ marine teleosts. It is critical in the care of aquarium animals that interdisciplinary groups communicate and coordinate animal care to maximize animal wellness outcomes. One example of this collaboration would be in assessing the feeding rate of a group-fed mixed-species exhibit based on animal health status, nutrient composition of the diet, and diet consumption by the animals. By collaborating on animal care, each group of specialists can offer feedback and unique perspectives on how their specialty can be leveraged to improve or enhance animal care.

This communication contains a brief review of some of the available literature that supports the summarized reports from each of the six working groups of subject experts. While some points are specific to the working group's specialty, others are multidisciplinary and will facilitate collaboration across disciplines. While working groups have some overlapping recommendations, their specific, individual perspectives and framing are considered valuable to each audience and retained rather than summarized. Each group has suggested goals for the next 3 to 5 years, with the ultimate objective of the workshop being the production of a husbandry manual for ex situ marine teleost nutrition and health.

CLINICAL MEDICINE AND PATHOLOGY WORKING GROUP

The fishes comprise a large, paraphyletic group with over 34,000 species (Froese and Pauly 2021). Marine teleosts are an important subset and include species that are important for public aquaria and the aquarium fish hobby, food, bait, research, and restocking of natural communities. Fishes collectively have diverse feeding strategies and correspondingly varied species-specific macro- and micronutrient needs. Basic nutritional requirements and related nutritional diseases are well documented in only a small number of commercially important species, most of which are reared in freshwater and used for human food fish production, with nutritional emphasis placed on attainment of rapid growth rates and efficient production (NRC 2011; Hoopes and Koutsos 2021). In 2016, aquacultured products contributed 46.8% of global seafood production (FAO 2018). We simply do not know which (if any) of these physiologic models that have been developed for commercially cultured food fish are suitable for the majority of marine teleost fishes that are maintained in zoos and aquariums. Furthermore, the production goals (reproduction and longevity) of exhibit fishes or those maintained for conservation purposes can differ drastically from those that are reared for aquaculture purposes. Nutritional requirements likely vary accordingly (Hoopes and Koutsos 2021). Feeding requirements of various life stages, particularly broodstock and larvae, are of particular priority for aquarium fish that are reared for display or to achieve conservation goals (Hoopes and Koutsos 2021). In contrast, nutritional requirements of juvenile fish during grow-out may be of greater interest for species that are cultured for food production. The use of low-cost diets, while optimizing survival and yield, is critical to managing production costs in aquaculture operations. Noninfectious and infectious diseases and syndromes may also have nutritional components (Blazer 1992; Davies et al. 2019), and these important interactions are largely unrecognized in the majority of aquatic species. Although some specific nutritional disorders are recognized, such as skeletal disorders, ascorbic acid, and thiamine deficiencies (Halver et al. 1975; Fitzsimmons et al. 2005; NRC 2011), many cases of nutritional disease are difficult to diagnose and only arrived at by exclusion. Hepatic lipidosis is an excellent example of a disorder that, while normal in some wild fish, is a common finding that is usually considered a pathology in animals under human care, particularly marine teleosts (Spisni et al. 1998; Wolf 2019). Despite being a frequent finding, the clinical significance of the condition is not always obvious (Wolf 2019). Additionally, fish that are maintained with suboptimal nutrition may present subtle anomalies that are characterized by poor growth, lack of vigor, poor reproductive performance, or increased susceptibility to infection (Davies et al. 2019). Thus, the role of nutrition, and even specific feed ingredients, is increasingly recognized as critical to optimal immune function and disease resistance (Zhao et al. 2015; Martin and Król 2017). Six complementary focus areas were identified by this working group as critical to improving the clinical recognition and management of nutritional disease in marine teleosts: (1) development of a standardized approach for antemortem (clinical) sample collection, (2) development of a standardized approach for postmortem (pathology) sample collection, (3) food biosecurity, (4) dietary supplementation, (5) application of advanced diagnostic methods, and (6) education and training in clinical fish nutrition.

Development of a Standardized Approach for Antemortem Evaluation and Clinical Sample Collection

During a clinical evaluation of a fish, morphometrics (including relative weight and body condition), the animal's general appearance, position in the water column, movement, and behavior have been used as a rudimentary proxy for nutritional status (Hoopes and Koutsos 2021). Occasionally, imaging or clinical pathology provide additional data; however, current levels of diagnostic investigation have limitations and can easily overlook subtle changes and effects to an animal's nutritional status. While radiology is an excellent tool for antemortem detection of skeletal and swim bladder anomalies (Soto et al. 2019), detecting liver pathology that is associated with nutritional abnormalities has been more dependent on necropsy findings and histopathology than premortem examination and clinical pathology (Wolf and Wolfe 2005). While some advances in fish health monitoring have been developed in aquaculture species—for example, automated blood cell count analysis (Fazio 2019)—widespread aquarium adoption has been slow. A more robust, standardized, and comprehensive approach for antemortem clinical evaluation is needed. This will require multidisciplinary collaboration among veterinarians, nutritionists, epidemiologists, aquarists, and systems engineers. Concurrently, standards of communication between fish suppliers and institutions need to be developed to ensure consistency of feeding, diet, and husbandry and to help with acclimation of wild or aquaculture fish into new environments. Both clinical evaluations and communication standards must remain flexible and evolve as new scientific information becomes available.

As part of this focus area, a baseline data set for nutritional disease needs to be developed. A number of questions must be addressed during this process, including those regarding (1) which diseases and disease processes can be accurately diagnosed and evaluated and (2) what samples and diagnostic tests are relevant and appropriate. For example, in addition to morphometrics, gross visual scoring, and standard clinical pathology, how can imaging (ultrasound, radiography, MRI, CT), metabolite and or microbiome evaluations, and other emerging technologies be standardized for various types of samples, validated, and prioritized considering resource limitations? Processes for evaluating functional feeds, defined as feeds that are supplemented with the intent of optimizing fish health as well as growth, need to be enhanced through the use of "omics" technologies to clarify molecular and cellular processes that are influenced by various additives (Martin and Król 2017). Similarly, species-specific baseline data (wild vs. captive, age-related, and sex-related) for feeding response behaviors as well as relevant organ and tissue samples must be gathered, standardized, and quantified. Approaches to best assess the nutritional status of mixed species tanks need to be established and evaluated.

Development of a Standardized Approach for Postmortem Sample Collection

A standardized process for postmortem evaluation and sample collection of a fish's nutritional and disease status will help inform clinical evaluation standards. Standardization will also provide more specific baseline data at both the gross and microscopic levels of structure. Likewise, diagnostic imaging, clinical pathology, and other morphometric and pathophysiologic indicators can be evaluated for their diagnostic or predictive value, especially in cases that are euthanized prior to necropsy. One example would be to develop a more specific and standardized definition of hepatic lipidosis, including gross, histologic, and clinical pathologic evaluation. As with clinical evaluations, postmortem standards must be flexible and updated over time as new information is learned.

Diet Item Biosecurity

Food biosecurity, defined here as the concern for accidental introduction of pathogens via food items, is another critical topic in need of further evaluation and standardization. Food items can serve as potential reservoirs for communicable pathogens such as viruses, bacteria, parasites, and fungi, but they can also contain heavy metals, microplastics, or adulterating ingredients. An example is liver pathology caused by aflatoxicosis resulting from the contamination of food stuffs with *Aspergillus flavus* (Frasca et al. 2018). How does food biosecurity affect gut health and immune function? This focus area requires multidisciplinary collaboration among nutritionists, collectors and distributors of food sources (live, frozen, and commercial feeds), aquarists, microbiologists, immunologists, and epidemiologists to summarize the current literature and determine best practices for (1) pathogen reduction/destruction, including irradiation, freezing, and other methods; (2) food handling and storage; (3) pathogen/microplastics/toxin testing; and (4) quality control.

Supplemented Feeds

A fourth relevant area of teleost nutrition in need of further investigation, expansion, and standardization is use of food for oral delivery of desired supplements and medication. Food is frequently used as a primary delivery mechanism for drugs, vaccines, and vitamin supplementation. Specific protocols for nutritional support are needed and may include the modification of delivery methods for diverse taxa and the use of food items for delivering feed additives, which could include specific vitamins or minerals, appetite stimulants, probiotics, or prebiotics (Hoopes and Koutsos 2021). The consideration of equipment, carrier methods (e.g., gel food, microencapsulation, and biodegradable gels), and administration logistics (e.g., target or tube feeding), as well as further investigation into the application of markers to ensure proper administration/consumption, is warranted. In addition to troubleshooting the practical approach to delivery, there is also a need to better understand vitamin and mineral requirements as well as drug pharmacokinetics, which likely depend on species, life stage, and reproductive status.

Advanced Diagnostics

While advanced diagnostic techniques may be a subsection of focus areas 1 and 2 (standardization of approaches for clinical and pathologic evaluation of nutritional disease), this topic is so broad, technical, and rapidly evolving that the authors believe it deserves special attention. As advanced human and domestic animal diagnostic approaches develop, progress, and become conventional, many of these methods become more readily adaptable and available for other species, including fish. Concurrently, many advances in teleost biology and physiology, environmental microbiology, and related fields may have clinical or pathologic applications. Areas that are currently underused in teleost clinical diagnostics and pathologic investigations include comparisons with established species-specific baseline values and health assessments using immunologic methods and species-specific markers (including evaluation of blood and mucus samples, genomics, transcriptomics, proteomics, metabolomics, and other relevant molecular markers). Additionally, the evaluation of microbial communities in the fish gut and any apparent alterations in response to different external and internal conditions should be considered. The role of gut microbes in digestion and nutrient availability is poorly understood in teleosts and is another area that is in need of investigation (Hoopes and Koutsos 2021).

Education and Training in Clinical Fish Nutrition

Tackling the basic knowledge gaps and developing standard approaches within the previously discussed focus areas are critical to the advancement of marine teleost nutrition. However, without targeted programs in education and training, the knowledge gained will not be disseminated effectively to all of the relevant stakeholder groups—including those at the front lines of husbandry and veterinary care. Coordinated collaboration among nutritionists, veterinarians, and husbandry staff is critical to success, as is proper identification and targeting of other stakeholder groups, such as collection fish wholesalers and producers, veterinary students, and diet item and feed suppliers.

NUTRITION WORKING GROUP

The nutrition working group consisted of participants from a wide range of backgrounds and expertise, including applied nutritionists and husbandry specialists for marine teleosts as well as commercial aquaculture species, nutritionists from commercial feed manufacturers and ingredient suppliers, and representatives from academia and conservation communities. The group recommended several areas of focus for which the current body of knowledge should be summarized and additional information be further developed. These areas of focus are detailed below and include creation of a husbandry manual containing practical feeding management information; a comprehensive review of current knowledge of nutrient requirements of marine teleosts; a summary and/or database of available food items, including assessment of their sustainability and considerations for food options for the future; and a review of current knowledge of larval and broodstock nutrition, as well as that specifically related to nutrition of herbivorous fish.

Practical Feeding Management

The nutrition working group recommends and supports the creation of a readily accessible husbandry manual to provide comprehensive information regarding practical feeding and nutritional management for marine teleosts under human care. Specific areas to address include techniques for nutrient delivery, including target feeding and methods for diet distribution and usage, and provisioning feed at an appropriate rate for the physiology and behavior of the target animal. Examples of this type of contribution can be found in many species survival plans as well as for some other aquatic species (e.g., elasmobranchs; Janse et al. 2004). Additionally, methodology is needed for health and welfare assessment in applied nutrition programs, including the development of behavioral tools to assess feeding response, body condition scoring methods, and tools for the estimation of biomass in large, mixed-species exhibits, which may be guided by behavioral work that was intended to facilitate other interventions (e.g., Corwin 2012). Finally, guidelines for food item and diet preparation are necessary, including methods for quantifying food items, frequency and types of analyses required, and standard procedures for maintaining food safety and quality through diet item delivery, storage, and feeding, such as recommendations for handling fish that will be fed to fish-eating animals (Crissey 1998) and for food preparation and feeding fish (Hoopes and Koutsos 2021).

Designing Feeding Programs for Marine Teleost Fish

The nutrition working group recommends a comprehensive peer-reviewed manuscript of the current knowledge of nutrient requirements and feeding programs for marine teleosts under human care. Specifically, this manuscript/review will summarize the current knowledge of nutrient requirements of various marine fish species to serve as a guide for diet development and assessment as well as integration of nutritional ecology knowledge to establish feeding guidelines for a species based on in situ cohorts, with the ultimate goal of using this expanded knowledge base to make actionable recommendations of feed management for marine teleosts. The vast majority of literature concerning the nutrient requirements of teleost fish has been generated in and for aquaculture species for which growth rate is often the primary variable by which titration of nutrient requirements is quantified. Thus, additional considerations for longevity, display needs (e.g., pigmentation of fish on display), reproductive success, etc. will have to be taken into consideration when applying and adapting data that are generated in aquaculture species. Additionally, a summary of best practices for animals with special dietary needs, including quarantine animals and animals with acute or chronic medical and rehabilitation challenges, will be important. A substantial portion of this information has recently been published (Hoopes and Koutsos 2021).

Diet Items for Marine Teleosts

The nutrition working group recommends the development of reference materials that summarize the availability of and opportunities for incorporation of various diet items for marine teleosts under human care. The working group recommends that the dissemination of this information include at least one peer-reviewed journal article and the establishment of a framework and protocol for an open-source, online database of foodstuff nutrient composition and a summary of the available diet items (current and historical), encompassing the broad range of fresh and frozen aquatic food items, supplements, and dry/prepared diet items that are typically used in marine teleost feeding programs. Additionally, the sustainability of these items should be assessed and improved. As a result of this workshop, research has been initiated to investigate the application of black soldier fly *Hermetia illucens* larvae meal as a sustainable replacement for fish meal in marine teleost diets with no significant differences in growth performance (S. Williams and coworkers, unpublished data). Other opportunities considered for future diet development (e.g., culture methods for live feed organisms, alternative sources of nutrients, improving formulation of diets for water quality, value of diversity in the diet) should also be investigated.

Larval and Broodstock Nutrition

The nutrition working group identified the critical need to facilitate breeding within marine teleost facilities and thus reducing the need for collection from wild fish stocks. The working group recommends the development of a summary of current knowledge of larval and broodstock fish nutrition, expanding on previous publications (e.g., Hamre et al. 2013). Recent successes with captive propagation of acanthurid, chaetodontid, and labrid fishes should be emphasized, with a focus on how these advances collectively contribute to the aquaculture of marine teleosts. A summary review article on this topic and contributions to an online database detailing foodstuff nutrient composition are anticipated outputs of this focus area, particularly identifying information gaps that can be targeted for future applied activities.

Specialty Diets Including Herbivorous Fish Nutrition

The nutrition working group recognized the need for a comprehensive review of the current knowledge of herbivorous fish nutrition, expanding upon work previously published (e.g., Clements et al. 2009). Recent work has improved our understanding of both feeding strategies and nutrient usage by a variety of herbivorous fish species; however, the majority of information available on the natural diets of herbivorous fish that graze on coral reefs amounts to little more than feeding observations. It is recommended that newer data sets, including biomarker data that identify actual dietary targets and specific assimilation of dietary elements, be a focus area of this review. A separate database detailing nutrient composition (and/or utilization) of foodstuffs consumed by this subgroup of fishes may be an additional useful output.

Overall, the nutrition working group identified these five areas for further summary and, ideally, additional research and future funding focus. Integration of current knowledge and recognition of data gaps will provide a foundation for future collaborations and targeted activities for greatest progress moving forward.

ANIMAL HUSBANDRY WORKING GROUP

A variety of methods are used for managing nutrition and delivering food to fish in large aquariums. These methods vary depending on species, age of individuals, fish population, facility preferences, behavioral husbandry application, exhibit size and shape, cohabitants in the exhibit, and feed type. Because of these distinctive variables, there is no simple solution for determining a best method that applies to every system. However, there is information, albeit limited and dispersed, on many strategies used in aquariums. Every aquarium team tends to develop its own set of institutional knowledge based on its physical facility, species, and staffing. Modifications of common feeding methods developed by on-site staff are often trialed before a specialized practice is developed for that particular operation.

Most of the peer-reviewed information on teleost nutrition is based on aquaculture species. This information is often based on economics and fast growth, thus not taking into account a balanced nutrition plan for all life stages and mixed-species habitats as we would see in aquarium settings. Applying this information for use in large aquariums containing various species and sizes of fish often requires modification of practices that were successfully used by aquaculture industries. Behavioral observations, health assessments, necropsies, and animal body scoring are all used as indicators of feeding strategy effectiveness and evidence that proper nutrition requirements are being met.

Body Condition Scoring

Applying techniques to evaluate body condition and feeding response of teleost fishes as a way to score the population is a newer practice within the industry. While detailed body scoring criteria have been developed for certain species of teleosts (Priestley et al. 2006) and elasmobranchs (Kamerman et al. 2017), they are limited in comparison to the mammalian, avian, reptile, and amphibian species resources available (AZA Nutrition Advisory Group 2021); such extensive resources do not yet exist for most teleost species. Further, the subjective nature of body condition scoring can be challenging due to differences in human perception; more work is needed to develop objective descriptions to improve body condition scoring accuracy relative to clinical evaluation.

Feeding Techniques

Traditional feeding techniques, designed to reduce competition between specific animals and/or species in a large multitaxa exhibit, can include broadcast feeding, location-specific feeding, simple target feeding, mechanical feeders, and the use of nonspecific diet items. To ensure that an adequate amount of diet is consumed, aquarists may feed a greater amount of food to multiple species at one time in a "broadcast" feed over a large surface area or choose to feed smaller amounts of food in specific locations targeted to certain animals or species. A disadvantage of broadcast feeding, whether in a large system or smaller "jewel" tanks, is the potential for more food waste by overfeeding or underfeeding certain individuals due to competition. Modified mechanical feeding devices such as pumping the food to underwater outlets and offering nonspecific diet items like lettuce in underwater feeders and bags can help disperse food at various depths and provide different feeding locations for a variety of species in large exhibits. Feeding a variety of taxa often involves various feeding groups (i.e., carnivores, herbivores, and omnivores) of fish. It is very important to be familiar with your collection and each species' life history to ensure that the fish are offered the correct diets in the correct manner. This is often overlooked in the development of feeding regimes. Knowledge of your collection is essential for development of effective diets and feeding methods. The development of specific care plans that are focused on caring for particular groups (genus or species, life stage, habitat) can improve the specificity of feed delivery and health outcomes.

While the Nutrition Working Group section of this manuscript has previously discussed nutritional areas of potential future of research, there is plenty of potential for improvement in husbandry feeding protocols as well. Record keeping within institutions and sharing techniques across institutions could lead to codification of best practices for many diverse species.

Behavioral Husbandry

Training husbandry behaviors is an essential part of well-rounded and excellent animal care programs, and it can provide animals with the mental stimulation, physical exercise, and cooperative veterinary attention and treatment they need to successfully survive in the environment that is provided for them in zoological settings (Ramirez 1999). Studies have shown that fish have demonstrated a high capacity for learning through observational, spatial, and aversion techniques (Helfman and Schultz 1984). The application of more advanced behavioral husbandry practices can be implemented to create a comprehensive and effective feeding strategy in even the most complex aquatic environments. By using operant conditioning techniques through positive reinforcement to modify behavior, aquarists have been able to condition animals (individuals or groups) to come to a recognized "target" (typically a discernable shape) or location for a feeding session. Target trained fish are often fed by hand or tongs, which allows for an exact amount of diet and supplements to be delivered. Many times, these animals are fed at the same time as others in an effort to keep them separated and prevent potential interruptions and/or competition with tank mates. Animals can also be trained to voluntarily move into a net or holding area for feedings (Corwin 2012). This not only eliminates competition and ensures accurate delivery of diets and supplements, but can also help set these animals up for future success in training them to participate in other aspects of their husbandry care.

As more innovative feeding methods for teleost fishes are designed and implemented, it will be vital for institutions to both share their trials and successes and promote industry standards for marine teleost nutrition. Currently, the details and complexities of providing for teleost nutrition in public aquariums are generally shared between facilities informally through conversations between colleagues, industry listservs, and annual conferences. To efficiently capture these information exchanges, share best practices, and archive them for the future, there is a considerable need for a single common electronic hub that can be easily accessed and used among institutions.

PHYSIOLOGY AND CHEMISTRY WORKING GROUP

The physiology and chemistry working group consisted of participants from a wide range of backgrounds and areas of expertise, including applied chemists, animal husbandry experts, and academic nutritional physiologists. After discussing the needs for understanding the aquarium environment and the physiological demands of the aquarium inhabitants, the group generated two focus areas that are critical for moving the field forward.

The Organic Matter Constituents of Dissolved Organic Matter

Using Fourier-transform ion cyclotron resonance mass spectrometry, it is possible to identify the number, and sometimes types, of compounds that exist in the dissolved organic matter (DOM) of seawater (Hansman et al. 2015). Seawater that is taken from the world's oceans is composed of tens of thousands of different compounds, whereas DOM from recirculating aquaria has significantly fewer (Semmen, unpublished data). Dissolved organic matter constituents may play a role in aquarium health, and thus we ask the following questions: what are the compounds composing the DOM of natural seawater (sensu Hansman et al. 2015), and what is missing in aquarium water? Do the constituents of DOM affect the "health" of the aquarium? This should be a primary area of research and will dovetail with microbial diversity and function. What roles do microbes in the aquarium environments play in DOM chemical diversity and variation? Which microbes matter more, those in the aquarium environment (including those associated with specific animals or plants) or those in the biological filtration systems? And finally, is sterilization with ozone/UV a good idea if beneficial microbes are killed in the process? Does this affect DOM concentrations and diversity?

Understanding the Needs of the Consumers within the Aquarium Environment

Each aquarium's physical environment will be different, and clearly not all community members can eat the same thing or require the same amount of space. By using energetics models (based on respirometry and accelerometry of at least closely related species to those in captivity [e.g., Parsons 1990] and digestibility estimates for different foods [German 2011]), different guidelines could be developed for each group or individual within a population. This requires significant work to be performed on many different species but with their management in mind. Data from aquaculture efforts cannot be easily extrapolated to aquarium fishes, especially because of the diversity of species kept in aquarium environments (aquaculture is focused on a handful of mostly carnivorous species; Clements et al. 2014). Thus, we are calling for deliberate studies of digestibility and energetic needs of as many species held in aquaria as possible to develop a better understanding of the true needs of each species. With these kinds of data, trophic guilds (Clements et al. 2017) can be identified for each environment based on their needs. Although covered in other sections (e.g., Nutrition Working Group), providing as realistic food as possible will be crucial for lower trophic level consumers (herbivores/detritivores). Some of the most visually dynamic members of aquatic communities, like herbivorous surgeonfishes, cannot subsist on diets of lettuce or kale alone, as these are terrestrial plants and surgeonfishes naturally graze or browse on algae (Choat et al. 2002, 2004); algae are very different from terrestrial plants in terms of nutrients and fiber (Choat and Clements 1998). What can be done to increase the utility of food for consumers that feed at lower-trophic levels?

We recommend a dedicated focus on determining the energetic needs of each species as well as the digestibility of different diets. Additionally, examining the potential of outdoor enclosures with naturally occurring algae would be a project idea. Is this sufficient to grow the requisite algal diversity (especially if the algal community is seeded with algae from the fishes' natural environment)? Should herbivorous/detritivorous animals be allowed time in outdoor enclosures as a means of increasing their access to algae as a food source, or is it sufficient to grow algae and biofilms in tanks that are exposed to sunlight (e.g., Hauter and Hauter 2019) and then bring these food items inside to offer to the consumers? Finally, in exploring the potential possibilities of feeding supplemented diet items, some experimentation with different compounds (like humic substances; Yılmaz et al. 2018) may help, although a natural diet and environment are best when feeding fish species. Matching the natural diet as much as possible is ideal, and meeting the energetic and nutrient requirements is critical. This is an area in which we currently are not adequate, especially for lower trophic level consumers in marine aquarium environments. However, we must work to improve managed diets if we cannot practically feed the natural diet (Yılmaz et al. 2018). Finally, ensuring palatability of offered diet items so as to avoid their degradation in the water due to nonconsumption is critical.

Overall, the limits of using recirculating systems are probably linked to water chemistry and microbes on some level (see the Water Quality Working Group section). However, attempting to understand the constituents of seawater beyond ionic or nitrogenous compounds (e.g., ammonium, nitrite, nitrate) will be critical in maintaining long-term recirculating systems. Moreover, improved tools are needed to improve our ability to truly understand individual animal performance within a larger community, improving our decisions regarding animal health and maintenance.

WATER QUALITY WORKING GROUP

Water is one of the richest and most diverse microbial reservoirs on Earth and it informs the biota of exposed fish tissues. Perhaps not surprisingly, the establishment of interactions with the water biota is critical for fish to adapt to many adverse aquatic environments. Like other animals, fish host and live among communities of microbes that influence a wide variety of their biological processes. Recent surveys of these healthy fish microbiomes have begun to document which species are present, how they facilitate fish health and functioning, and the role of water quality in selecting, promoting, and controlling them. More comparative studies are needed to determine whether characteristics such as nutrient and mineral availability are major determinants of the fish microbiome. Just as digestive tract microbes interact with the food consumed that is by terrestrial vertebrates, the fish gut and gill microbiomes mediate the aquatic-based diet and nutrient ion exchange.

Microbial Diversity

Microbial diversity is influenced by environmental complexity. The density of microbes in many aquatic systems is staggering, with tens of millions of organisms and thousands of species per liter having been described (Sogin et al. 2006). For example, seasonal time series analysis revealed repeated annual patterns in marine microbial communities off the coast of San Pedro California (Fuhrman et al. 2008). These repeating patterns indicate that environmental parameters are ecological drivers that shape marine microbial communities, and they include chemical and physical parameters such as temperature, pH, nutrients, and salinity (Van Bonn et al. 2015). On the other hand, water treatment processes in artificial systems maintain the chemical and physical values within a restricted range of variation and their management typically includes reduction in microbial abundance. As a result, it is likely that the microbial assemblages in aquarium systems differ significantly from natural systems, with the former having lower microbial diversity. This makes niche spaces available for potential pathogens and potentially a reduced immune system memory for resident animals. Microbes found in managed aquatic systems are subject to powerful selection pressures. The equilibrium state of managed systems is characterized by a much different microbial ecology than naturally occurring systems (Vadstein et al. 2018). This, in turn, may have a profound effect on the adaptive immune responses of host organisms sharing the environment. For these reasons, disinfection and water conditioning practices and procedures for aquarium systems should be reviewed.

Disinfection

The disinfection of managed systems is used to reduce infectious organisms as well as to control undesirable algae and color of the water column and along surfaces. It is a "point source" process, as the inline application of ozone during filtration dissipates before it gets into the habitat. Advances in disinfection processes, such as more efficient ozone mass transfer, automated control systems, and less disruptive ozone contact used in combination with foam fractionation, help mitigate some of the undesirable effects of residual oxidants. We are proposing that, in light of recent findings, it is time to eliminate the word "disinfection" from our aquarium vocabulary.

There are indirect effects on health caused by the disruption of the fishes' host and environmental microecology that are brought about by oxidation disinfection. There is literature that suggests that ozone could also be affecting fish health indirectly by transitioning metal oxidation to a more biologically reactive valance state of iron (Bagnyukova et al. 2006), copper (Craig et al. 2007; Bopp et al. 2008) and chromium (Lushchak 2008; Lushchak et al. 2009a, 2009b; Kubrak et al. 2010; Vasylkiv et al. 2010), which leads to oxidative stress. In some cases, this process may be converting trace metal nutrients into toxic ions. There is evidence that ozone or its derivative byproducts might cause oxidative stress directly in fish as well (Fukunaga et al. 1999; Hébert et al. 2008). The totality of environmentally induced oxidative stress on an organism has been shown to add an accumulative oxidative stress effect on the animals' physiology, resulting in a stabilized, prolonged "quasi-stationary" state (Lushchak 2011). This could help explain why certain fish species, especially within the elasmobranchs, are more easily pushed over the edge with ozone-produced total residual oxidants in the aquarium environment than teleosts (Rudneva et al. 2014).

Managing the Microbiota

Current research provides a thorough description and characterization of gut microbiomes of aquaculture species (Wong and Rawls 2012; Llewellyn et al. 2014; Trinh et al. 2017). Changes that these communities exhibit when pre- or probiotics or other feed additives are incorporated into the diet have been documented (Abdel-Wahab et al. 2012; Sihag and Sharma 2012; Karlsen et al. 2017). Humic substances, when added to their water or diet, may help the animal's defense system by inducing a number of nonspecific host immune responses. These include the elimination of metal bioaccumulation in fish tissues. Also observed are increased production of biotransformation enzymes and stress defense proteins, such as chaperons or heat shock protein in fish and invertebrates (Menzel et al. 2005; Abdel-Wahab et al. 2012). In a study featuring Kelp Grouper *Epinephelus bruneus*, the addition of 1.0% chitin or chitosan extracted from shrimp shells to the diet stimulated immune response and enhanced disease resistance against infections of the protozoan parasite *Philasterides dicentrarchi* (Harikrishnan et al. 2012). Prebiotics and probiotics can also lead to health-promoting postbiotics, generated by a healthy gut microbiota that metabolizes ingested food to produce various beneficial postbiotic compounds. From human gut microbiota studies, we know these might include amino acids, vitamins, and short-chain fatty acids and may be anti-inflammatory, immunomodulatory, antiobesogenic, antihypertensive, hypocholesterolemic, or antiproliferative and may enhance antioxidant activities (Shenderov 2013; Sharma and Shukla 2016).

Microbial Maturation

Many studies on the effects of prebiotics and probiotics on farm-reared fish and their associated microbiomes have been published in the past decade (Goldin and Gorbach 2008; Sihag and Sharma 2012). This includes a new way of thinking about how hygienic barriers (e.g., antibiotic regimens, ozone and UV disinfection), organics, and other nutrients are managed to allow for the microbial maturation of water and systems (Attramadal 2011; Attramadal et al. 2012). Defined in part as the selective promotion of slow-growing competition specialists, the K-strategist bacteria (Skjermo et al. 1997; Salvesen et al. 1999; Skjermo and Vadstein 1999; De Schryver and Vadstein 2014) are assumed to act as a barrier against pathogenic invasion and establishment by opportunistic r-strategists (Stecher and Hardt 2008). In the natural environment, heterotrophic bacteria obtain part or all of their carbon (C) resources from algae (phototrophs), thus making this interdependence between bacteria and algae inseparable. At the same time, heterotrophs are competing with algae for the available reactive phosphorus (Ortho-P) in the water column and competing with them for biofilm recruitment space. Therefore, a healthy biofilm community, characterized by a high diversity and stability, is dependent on a healthy ratio of heterotrophic to phototrophic microorganisms. This is dictated by the C:P ratio (determined to be around C:P = 1) in the water column and controlled niche spaces or surfaces (Hall and Pepe-Ranney 2015).

Application

We must keep in mind we are feeding not only the fish host, but also its symbiotic microbiome. Microecology principles dictate feeding as close to a natural diet as possible. Fresh and raw foods are good, but facilitating the feeding of living biota could go beyond improved nutritional content to the infusion of natural probiotics and prebiotics. These are critical to the host's utilization of the food's nutritional content, including naturally occurring extracellular polymeric substances, chitin, and humic substances. Enriching the water might include the addition of water-conditioning probiotics and prebiotics routinely to avoid r-strategist takeovers, tapering of traditional disinfection and organic matter control, and a more ecologically diverse biofauna. The latter might be accomplished by incorporating a diurnal rotation routine by moving water and fish through interconnected microcosm modules with environments and substrates that facilitate the culturing of biota native to the fishes' grazing environments.

EPIDEMIOLOGY

The epidemiology working group concluded that there is a critical need to first design and distribute a survey to collect critical information that will facilitate the assessment and evaluation of factors that affect teleost health. It is important to be able to accurately quantify disease status and risk factors (including the environment) that are associated with individual and population teleost health. A consensus was reached among all participants in the workshop regarding the need to gain knowledge for different teleost species and life stages. General approaches and considerations to address current challenges on teleost health were outlined by the epidemiology group and subsequently complemented by specific questions arising from each working group.

General Approaches and Considerations

It was agreed that species-specific diet requirements are needed as well as behavioral standards and better knowledge of environmental conditions, including water quality. Understanding changing nutritional, behavioral, and clinical demands at different life stages within species was also highlighted as an important area of investigation. The quality and quantity of records/data available are a primary consideration when moving forward and trying to better understand teleost health. Procedures for obtaining future data will be important, but access to centralized resources of currently available data would be beneficial as well.

The "unit of interest" (tank, population of certain species, individual fish) needs to be clearly determined when assessing teleost health. At the same time, it is important to identify parameters and approaches that can be measured and used effectively for multiple species (i.e., delivery of food and managing feeding behavior). These should be differentiated from instances in which species- or age-specific approaches will be more appropriate. Although sometimes the goal is not to compare ex situ populations to wild ones, it is important to have reference data from wild populations to understand baseline parameters. The biggest challenge in the care of ex situ marine fish is to establish more complete definitions and standards for animal health, reproduction, nutrition, environment, and behavior. Thus, each working group developed central questions that will serve as the foundation for developing the proposed survey.

This group thinks that it is extremely important to address current gaps in knowledge regarding teleost health and nutrition to better address the challenges faced by ex situ teleost populations. Collecting and analyzing this information will be a first and critical step toward having a positive effect in maintaining the health of ex situ teleost populations worldwide.

CONCLUSION

In analyzing the framing that is used by media outlets when discussing zoos and aquaria, we found that institutions can be viewed in many contexts, with animal welfare, business interests, and their function as entertainment/recreation accounting for 85% of the articles that we studied. Additionally, while a majority of these media articles were supportive of zoos and aquaria, the articles that were negative were overwhelmingly focused on animal welfare topics (Maynard 2017). This growing focus on animal welfare among the public is an example of why scientists and animal care professionals must continue to collaborate and engage in science to improve the care of our aquaria species. The importance of animal welfare to the public and the ability of zoos and aquaria to affect the public show how excellence in animal care directly influences guests' perception and the institution's ability to educate the public and advance their conservation efforts.

The goals and areas of focus outlined above represent information that needs to be expanded and developed over the coming years to provide a strong foundation for the production of a husbandry manual for marine teleost nutrition and health. Much of the existing knowledge in teleost health and nutrition is focused on production aquaculture, which seeks to maximize outputs while minimizing input costs. In aquaria, our goals are often the opposite, focusing on maximizing life span and maintaining animal health with vibrant coloration. Our goal in developing a robust animal care manual with a multidisciplinary focus is to continue the improvement in the care and condition of our collection species. It is important to consider all aspects that affect fish health and wellness when designing an animal care plan, including clinical medicine, nutrition, animal husbandry, and water quality. Enhancing our knowledge of both in situ and ex situ systems will improve our understanding of how fish interact in complex environments and better support their diverse requirements in aquaria settings. By enhancing our animal care practices, we can better serve both our local communities and our conservation and education goals as zoo and aquaria. Through fostering a strong communication network among fish professionals, we hope to gain greater insight on best practices as well as emerging science to drive innovation and excellence in teleost care. By collaborating across institutions and disciplines, we hope to promote and enhance the welfare of fishes under human care through improved nutrition and health.

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