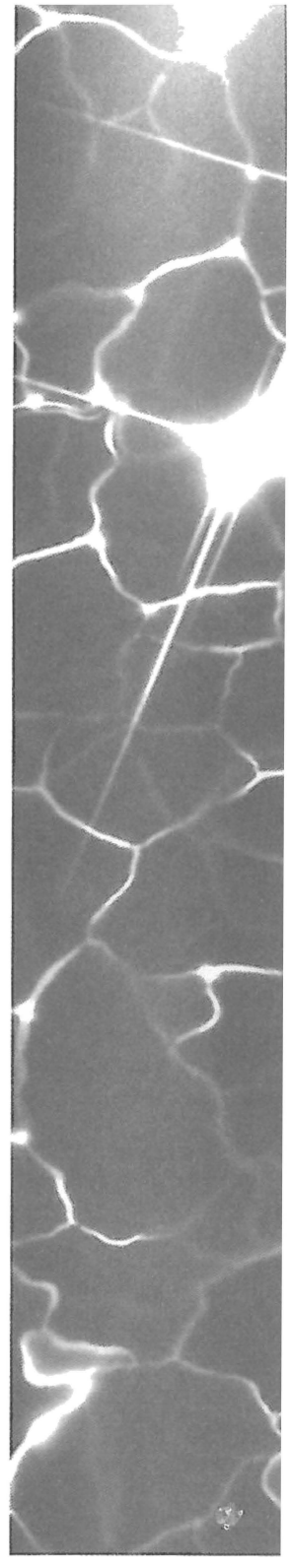
International Journal of Recirculating Aquaculture



June 2003
Volume 4

VSG-03-18

Printed in the United States of America

ISSN 1547-917X

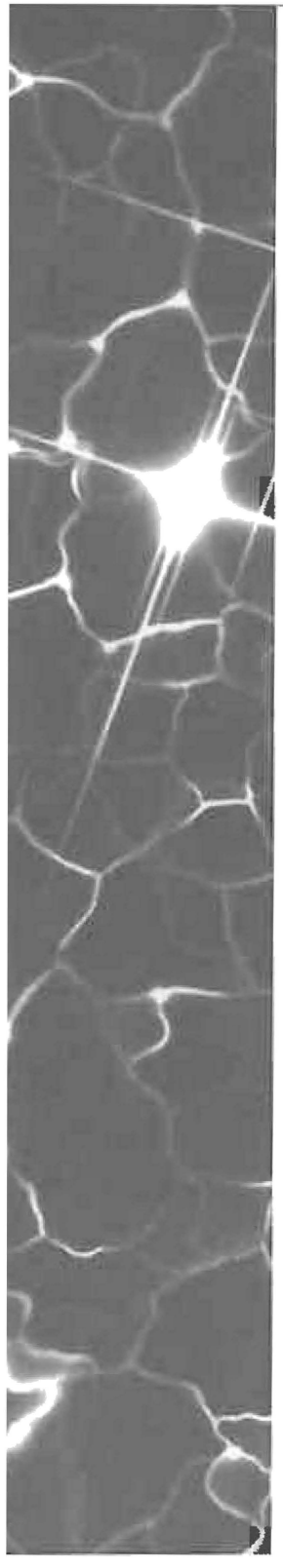
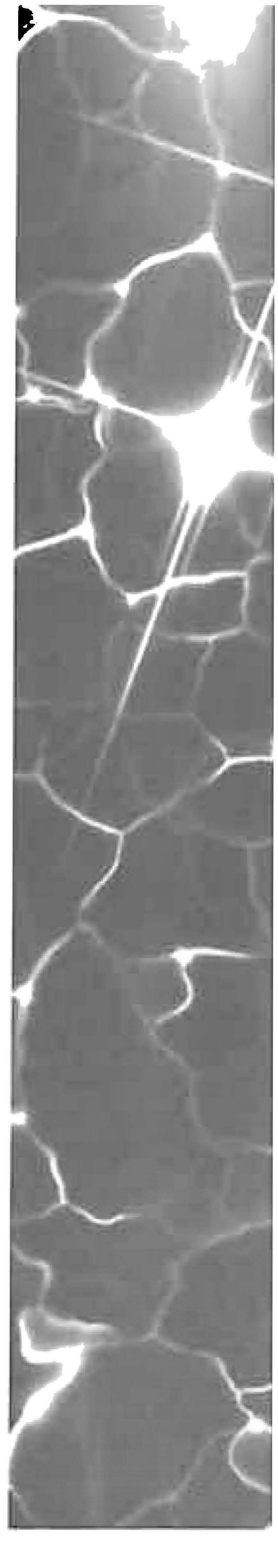


TABLE OF CONTENTS
(Volume 4)



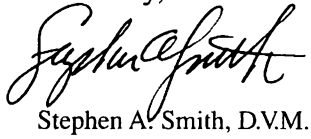
Dear Reader:

As the *International Journal of Recirculating Aquaculture* completes its fourth year of publication, two important new opportunities have opened up for us. First, articles published in the *IJRA* are now able to get listed with the major literature citation and abstracting services both in the U.S. and internationally. This means that our journal, including its articles and authors, will be fully searchable, and that papers published in the *IJRA* will be available for use by aquaculturists and researchers all over the world.

Second, we will be sending a complete set of our published volumes to libraries all over the world, increasing the availability of the journal for aquaculturists, researchers and students. We will continue sending the journal to university libraries and aquaculture/agriculture libraries on our list as soon as new issues become available. We will be working to make sure that both of these new avenues yield benefits for both our readers and our authors.

We encourage aquaculturists working with recirculation technology, whether in academia or the private sector, ponds or tanks, indoors or out, to send us manuscripts in the areas of water conservation, systems management, waste management, species selection and evaluation, breeding and genetics, larval rearing, nutrition, fish health, aquatic medicine, food or product quality, food safety, and marketing and economics. Our broad-based journal is tailored to serve our diverse readership, bringing thought-provoking and useful research into wider use.

Sincerely,



Stephen A. Smith, D.V.M., Ph.D.
Executive Editor *IJRA*

Characteristics of the Recirculation Sector of Finfish Aquaculture in the United States and Canada

J. Delabbio[1]*, B. R. Murphy[1], G. R. Johnson[2], E. Hallerman[1]

[1] Virginia Polytechnic Institute and State University
Dept. Fisheries and Wildlife Sciences
Blacksburg, Virginia 24061-0321 USA

[2] Fish Pathology Unit, Atlantic Veterinary College,
University of Prince Edward Island
Charlottetown, PEI E0G 2X0, Canada

* Corresponding author, current address:
Bluefield State College
P.O. Box 286
Bluefield, West Virginia 24701 USA
E-mail: jdelabbio@bluefield.wvnet.edu

ABSTRACT

In the autumn of 2001, a survey was conducted to examine basic farm production and human resource characteristics of recirculation facilities in the United States and Canada currently growing finfish. An 86% response rate was achieved. The survey data indicate that this sector of aquaculture is quite heterogeneous. The number and pounds of fish produced is quite variable, with presence of small-, medium- and large-sized farms in this sector. Recirculation technologies are employed to culture a wide variety of both warmwater and coldwater fishes in both saltwater and freshwater situations. The four fishes most commonly grown in recirculation units in the United States and Canada are Atlantic salmon smolts, tilapia, hybrid striped bass and ornamental fishes. A high proportion of facilities using recirculation technologies use pumped groundwater as a primary water source. Over 40% of facilities represented in the survey rely on a single water source to sustain their operation and have no secondary water source as backup. Management personnel of recirculation facilities are highly educated; more than 74% of respondents reported holding at least an undergraduate degree. The majority of personnel managing recirculation facilities are middle-aged individuals who have over 10 years of related work experience. The

findings of this study represent the first empirical description of the recirculation sector of finfish aquaculture in the United States and Canada.

INTRODUCTION

Over the past 25 years, aquaculture has been one of the fastest-growing sectors of US agriculture. In 1974, the farm gate value of all US aquaculture products was $45 million; by 1998, it had increased to almost $1 billion (USDA 2000). The 1998 Federal Census on Aquaculture (USDA 2000) indicated that there were over 4,000 aquaculture operations in the United States. These operations represent all sectors of aquaculture production (mollusks, finfish, crustaceans and plants). Many of these operations are quite small. Almost 50% of the aquaculture operations in the United States have an annual income of $25,000 or less (USDA 2000). These census data show that only a small percentage of the total US aquaculture industry currently uses recirculation technology. Of the 4,000 operations in existence, only 328 US fish farms identify themselves as "recirculation" facilities. However, due to the use of unclear terminology in the census, this number may be inflated and therefore misleading. The definition identifying recirculation operations was "reuse of water in an aquaculture facility (closed system) rather than releasing into nature and continually being replaced by new water (open system)". The use of this definition allowed a variety of activities that are pseudo-aquaculture in nature to be included under the recirculation category. For example, among the 328 recirculation facilities identified were operations run by fishermen to hold captured wild crustaceans during market lulls and when the animals are pre- and post-molt (and therefore less marketable).

The Canadian aquaculture industry also has grown substantially over the past two decades. Between 1984 and 1995, Canadian aquaculture production increased at an annual rate of 21.6% (FAO 1997). The Canadian Aquaculture Industry Alliance (CAIA) predicted that finfish producers will double 2001 production by the year 2006 (OCAD 2001). Similar to the US industry, the recirculation sector of the Canadian aquaculture industry lacks quantitative and qualitative description.

METHODS

A survey instrument was developed and administered to gather baseline information about facilities using recirculation technology in the United States and Canada. For this study, the defining element for a recirculation facility was that the operation "had to use a biofilter in its fish culture system". Information on currently operating recirculation facilities was obtained from three different and independent sources:

(1) government aquaculture representatives for each state and province,

(2) representatives from national associations representing particular sectors of aquaculture, and

(3) feed company representatives and research groups who have professional interaction with the recirculation sector of aquaculture.

Information from the three sources was cross-referenced to develop a final mailing list representing the current status of this sector. The initial sampling frame consisted of 162 facilities.

In spring 2001, the survey questionnaire and mailing protocol were developed using a modified version of Dillman's Total Design Method (TDM) (Dillman 1978). The questionnaire was pre-tested with six different managers of finfish rearing units in the United States and Canada. These managers represented different components of the finfish sector (business, research and demonstration facilities) and were asked to complete the questionnaire, give detailed comments on areas for improvement, and identify areas of ambiguity. Following the pre-test, the questionnaire was revised, printed, and mailed to 162 facilities in the United States and Canada. The questionnaire was composed of 43 questions. Three consecutive mailings were made during the fall of 2001. Each mailing included a cover letter, the complete questionnaire, and a stamped, return envelope. An incentive (a cookbook of farmed trout recipes) was offered to those respondents who returned the questionnaire promptly. Respondents were identified by a randomly-assigned number only.

Data Analysis

Response data were analyzed using Statistical Package for Social Sciences (SPSS, version 11.0, SPSS Inc., Chicago, IL, USA) software. Most of the data collected through the survey were nominal in nature;

frequency distributions were constructed for responses to all questions within nominal response categories. There were four continuous variables in the results; frequency distributions and other summary statistics were produced for these variables.

RESULTS

Overall, there was an 86% response rate to the survey. Correctly identifying the target people (names and addresses) before the first mailing of the questionnaire was key to achieving the high response rate of this study. One hundred and forty-one completed questionnaires were returned, but 10 of the respondents did not use a biofilter in their operation, and therefore did not fit the study's defining frame of a "recirculation" facility. The following results, therefore, incorporate data obtained from 131 facilities in the United States and Canada. Seventy-one percent of the respondents were from the United States (n=93) and 29% were from the Canada (n=38). The data represent information obtained from facilities in 32 states and 8 provinces.

Production Characteristics of Recirculation Units

The recirculation sector of aquaculture is composed of small-, medium- and large-sized farms (Figure 1). A breakdown of the sector by size of production (annual volume of fish) shows that the majority of recirculation operations (58%) produce 22,679.6 kg (50,000 lbs) or less of fish per year. Medium-sized farms (producing >22,680.1 kg [50,000 lbs] to 113,398.1 kg [250,000 lbs] of fish per year) comprise 25% of the sector. The remaining 17% of the farms are large farms that individually produce over 113,398 kg (250,000 lbs) of fish per year. Five very large farms (annual production greater than 453,592.4 kg [1 million pounds]) responded to the survey. Two of these farms produce tilapia (*Oreochromis spp.*), and one each grow Atlantic salmon (*Salmo salar*), chinook salmon (*Oncoryhynchus tchawytscha*), and hybrid striped bass (*Morone sp.*). Annual production expressed as number of fish produced was similar to production by weight (Table 1). Small-sized farms, producing fewer than 50,000 fish annually, comprised 41% of the respondents, medium-sized farms, those producing 50,000 to 500,000 fish per year comprised 26% of the respondents, and large-sized farms, those growing more than 500,000 fish per year represented 28%.

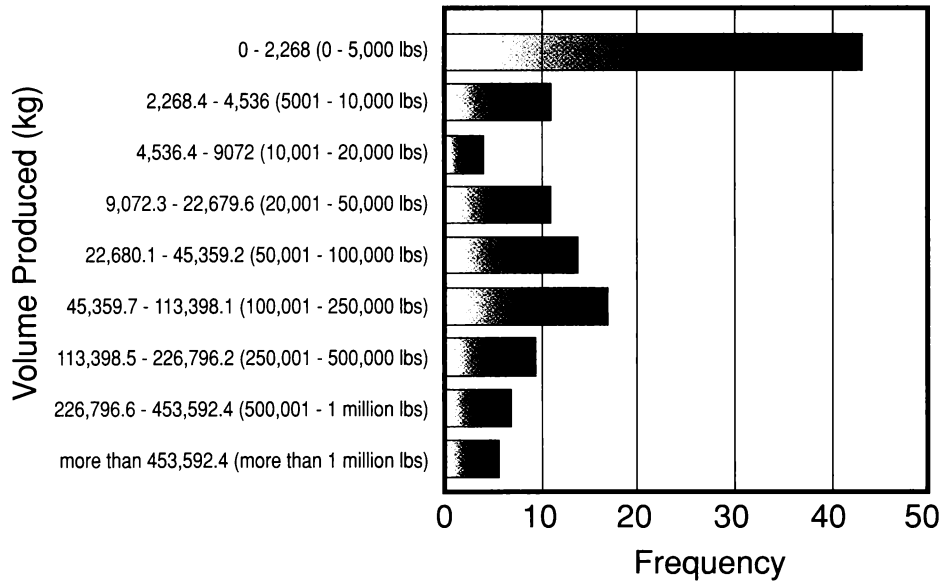


Figure 1: Annual production (kg) for recirculation facilities in the United States and Canada.

Fish Produced	Count	Percent	Cumulative Percent
0 - 10,000 fish	31	24.4	24.4
10,001 - 20,000 fish	7	5.5	29.9
20,001 - 50,000 fish	14	11.0	40.9
50,001 - 100,000 fish	7	5.5	46.5
100,001 - 250,000 fish	12	9.4	60.6
250,001 - 500,000 fish	14	11.0	71.7
500,001 - million fish	12	9.4	81.1
1 million - 1.5 million fish	12	9.4	90.6
more than 1.5 million fish	12	9.4	100.0
Total	127	100.0	

Table 1: Annual numbers of fish produced in recirculation facilities in the United States and Canada

Eighty-five percent of recirculation facilities were freshwater operations (Figure 2). The primary water source for 48% of the respondents was well water. Chlorinated municipal water was the second most common primary water supply (24%) for recirculation facilities. The respondents were also queried regarding secondary water sources used during a production cycle. Well water (28%) and chlorinated municipal water (18%) were the most commonly used secondary water sources for recirculation facilities (Table 2). Surface water was used by 14% of the respondents as a secondary water source. However, over 40% of the facilities used only a single water source and did not have a secondary water source for use in times of emergency.

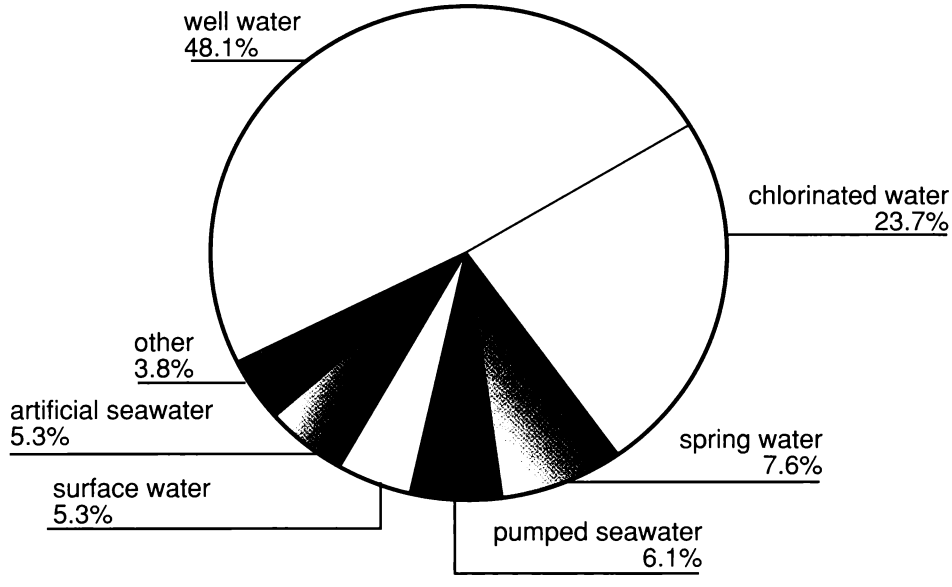


Figure 2: Main source of water for recirculation units in the United States and Canada.

Sixty-two percent (62%) of respondents used recirculation technology for business purposes, i.e., for profit-oriented production (Table 3). At present, recirculation technology is not frequently used for education (8% of respondents) or for demonstration (3%) purposes. However, the survey population included only college and university aquaculture programs and did not include secondary schools. We recognize that many secondary schools use small-scale recirculation systems in their natural science and agriculture curricula (there are over 20 of these units in secondary schools

Source of Water	Count	Percent Responses	Percent[1] Cases
well water - fresh	34	23.3	27.6
chlorinated water	22	15.1	17.9
surface water	17	11.6	13.8
artificial saltwater	6	4.1	4.9
pumped saltwater	5	3.4	4.1
spring water	5	3.4	4.1
reverse osmosis	4	2.7	3.3
saltwater well	3	2.1	2.4
only one water source	50	34.2	40.7
Total responses	146	100.0	118.7

[1] *Respondents were allowed to report more than one answer. Indicated percent represents the proportion of respondents reporting each particular answer.*

Table 2: Secondary source of water supply for recirculation facilities in the United States and Canada.

Purpose	Count	Percent
Business	81	61.8
Research	37	28.6
Education	10	7.6
Demonstration	3	2.3
Total	131	100.0

Table 3: Main purpose of recirculation operations in United States and Canada.

in the State of West Virginia alone; (Don Michael, WV Dept. of Education, personal communication). However, these secondary school units have little or no production output, and many are not functional year-round; thus, they were excluded from the survey population.

For respondents who indicated that business was the main purpose for using recirculation technology (Table 4), the most common business activities were: 1) growing fish for the food market (59%), 2) growing fish for sale to other farmers for grow-out (46%) and 3) supplying fish eggs to others (21%). Under the heading "other business activities", public aquaria, education outreach, and supplying fry to others for grow-out to smolt stage were each identified more than once.

Business activity	Count	Percent [1]
Grow food fish to market size	51	58.6
Supply fish for others to grow-out	40	45.5
Supply fish eggs to others	18	20.7
Grow fish for the ornamental market	14	16.1
Grow fish for stocking in natural waters	11	12.6
Grow fish for the bait market	2	2.3
Other	10	11.5

[1] *Respondents were allowed to mention more than one answer. Indicated percent represents the proportion of respondents mentioning each particular answer.*

Table 4: Types of business activities using recirculation technology in the United States and Canada.

The four fish types most frequently grown in recirculation facilities in the United States and Canada are tilapia (15%), Atlantic salmon (13%), ornamental fishes (9%) and hybrid striped bass (8%) (Table 5). Collectively, these four fish types constitute 45% of the sector. Fishes belonging to the family Salmonidae represented almost 28% of the fish taxa grown as the primary crop in recirculation facilities. Seventeen different categories of fish were identified in the questionnaire, plus a write-in area to report on other fish types not mentioned in the questionnaire. In the "other" category (n=48), a wide variety of different species were reported, including some saltwater species (Table 6).

When operators were asked to identify which taxa of fishes they had grown in the past, more facilities had grown rainbow trout (*Oncorhynchus mykiss*) than any other fish type (46% of cases) (Table 7). Tilapia was the second most common fish type grown in the past (39% of cases).

The three life stages most commonly reared in recirculation operations (Table 8) were fingerlings (96%), fry (77%), and eggs (73%). Smolts constituted the smallest percentage of life stages grown (27%), but this is not surprising since this life stage is specific only to the group of facilities that grow salmonids.

Many facilities did not know the survival rates of the earliest life stages, but they did have this information for later life stages (Table 9). Overall, the highest rates of survival were found in the later life stages. From fry to

Name	Count	Percent Responses
Tilapia spp.	26	14.8
Atlantic salmon	23	13.1
Ornamental fishes	15	8.5
Hybrid striped bass	14	8.0
Arctic charr	9	5.1
Flatfish	9	5.1
Rainbow trout	8	4.5
Yellow perch	8	4.5
Brook trout	5	2.8
Chinook salmon	3	1.7
Sturgeon	3	1.7
Brown trout	1	0.6
Catfish	1	0.6
Baitfishes	1	0.6
Bass - LM and SM	1	0.6
Sunfishes	1	0.6
Others	48	27.3

Table 5: Types or species of fish currently produced in recirculation facilities in the United States and Canada.

Freshwater species		
lake whitefish	white bass	carp
hybrid carp	cobia	grass carp
walleye	coho salmon	striped bass
tiger trout	white seabass	pacu
paddlefish	bonytail	Rio Grande silvery minnow
razorback sucker	mummichog	Japanese medaka
Marine species		
cod		
sea bream		
haddock		
Asian catfish		
muttonfish		
black sea bass		

Table 6: "Other" fishes grown as primary production (fish crop) in recirculation facilities in the United States and Canada.

Name	Count	Percent Responses	Percent [1] Cases
Rainbow trout	36	12.0	46.2
Tilapia spp.	30	10.0	38.5
Ornamental fishes	26	8.7	33.3
Hybrid striped bass	21	7.0	26.9
Catfish	19	6.3	24.4
Atlantic salmon	17	5.7	21.8
Sturgeon	16	5.3	20.5
Sunfish	16	5.3	20.5
Brook trout	15	5.0	19.2
Yellow perch	15	5.0	19.2
Baitfish	14	4.7	17.9
Bass- LM and SM	14	4.7	17.9
Arctic charr	14	4.7	17.9
Chinook salmon	8	2.7	10.3
Flatfishes	8	2.7	10.3
Brown trout	5	1.7	6.4
Chum salmon	2	0.7	2.6
Others	24	8.0	30.8
Total responses	300	100.0	384.6

[1] *Respondents were allowed to mention more than one answer. Indicated percent represents the proportion of respondents mentioning each particular answer.*

Table 7: Fishes that have been grown in the past in current recirculation units in the United States and Canada.

Life stage	Count	Percent of Responses	Percent [1] Cases
Egg	94	14.7	72.9
Fry	99	15.4	76.7
Fingerling	124	19.3	96.1
Smolt	34	5.3	26.4
Yearling	68	10.6	52.7
Adult fish	83	12.9	64.3
Food market-sized fish	69	10.8	53.5
Broodfish	70	10.9	54.3
Total responses	641	100.0	496.9

[1] *Respondents were allowed to mention more than one answer. Indicated percent represents the proportion of respondents mentioning each particular answer.*

Table 8: Life stages grown in recirculation facilities in the United States and Canada.

market-sized fish, over 50% of the respondents reported survival rates of 90% or greater. Survival rates of the different life stages is a point of sensitivity both from a business performance standpoint and in terms of the fish culture ability of personnel. Therefore, it was not surprising that 38% of the respondents chose not to answer this particular question.

Use of recirculating technologies to grow fish is not limited to "new" facilities. The mean number of years of operation for the different farms responding to the survey was 11. The most frequently reported age of a facility was 6 years. However, it is important to note that the question on the survey did not ask the length of time that the operation had been using recirculation technology, but rather asked the number of years the facility had been operating. This wording was purposeful; many facilities embrace recirculation technologies in a step-wise fashion over a fairly long time. Therefore, the data on years of operation is indicative of the longevity of operation of the facility rather than the history of its use of recirculation technology. It is interesting to note that both old and new operations are using the technology and that age of the facility was not necessarily a criterion or obstacle to employment of the technology. However, newer facilities more frequently are using recirculation technology.

Characteristics of personnel of recirculation units

One purpose of the survey was to describe key characteristics of personnel managing recirculation units in the United States and Canada. A number of survey questions were directed towards characterizing staffing and personnel attributes.

Seventy percent of recirculation operations had 1 to 8 full-time employees. The most common situation in recirculation units is to have one full-time employee, but there is a broad range of staff size for full-time employees among the different facilities (Table 10). Most operations had a low number of part-time staff (the modal number of part-time staff employed at recirculation facilities in the United States and Canada is 2). Sixty-six percent of operations had 1 to 5 part-time staff (Table 10).

Overall, the majority of managers operating recirculation facilities in the United States and Canada are middle-aged, highly educated and well-experienced in aquaculture. Manager's ages ranged from 22 to 72 years (Figure 4) . The average age for a manager was 43 years (mode = 45

	Rate of Survival											
Life stage	**More than 90%**	**90%**	**80%**	**70%**	**60%**	**50%**	**40%**	**30%**	**20%**	**10%**	**Less than 10 %**	**Not known**
From fertilized eggs to eye-up	12.3	17.3	18.5	13.6	6.2	4.9	1.2	1.2	—	—	—	24.7
From eyed-egg to hatch	23.0	23.0	14.9	11.5	3.4	2.3	1.1	—	—	—	—	20.7
From hatch to first feeding	23.1	22.0	11.0	9.9	5.5	3.3	5.5	2.2	—	—	—	17.6
From first feeding to fry	24.1	20.7	17.2	8.0	5.7	4.6	1.1	1.1	2.3	1.1	—	13.8
From fry to fingerling	35.8	15.8	11.6	8.4	4.2	5.3	2.1	2.1	1.1	2.1	—	11.6
From fingerling to yearling (smolt)	40.4	23.1	15.4	6.7	1.0	1.9	1.0	2.9	—	—	1.0	6.7
From yearling to market	43.8	24.7	9.0	5.6	—	—	—		3.4	—	1.1	9.0
From market to broodfish	39.1	18.8	10.1	2.9	—	—		1.4		2.9	2.9	21.7

Table 9: Average rates of survival reported for life stages held in recirculation facilities in the United States and Canada.

— Indicates no response in this category.

Number of Staff	Full-time Count	Full-time Percent	Part-time Count	Part-time Percent
0	8	6	18	15
1 – 5	74	59	80	66
6 -10	25	20	15	12
11 -15	10	8	4	3
16 - 20	5	4	3	2
> 21	4	3	2	2
Totals	126	100	122	100

Table 10: Number of employees in recirculation facilities in the United States and Canada.

years). Greater than 74% of the respondents had an undergraduate degree or higher level of education (Table 11). Personnel managing recirculation units have a considerable amount of practical experience in aquaculture as well as a high level of formal education. The managers who responded to this survey had on average 15 years of experience working in aquaculture, with the majority of managers having between 15 to 25 years of work experience.

Level of Education	Count	Percent	Cumulative Percent
Less than high school	2	1.5	1.5
High school diploma	8	6.2	7.7
Some college	9	6.9	14.6
Community College graduate	15	11.5	26.2
Bachelor's Degree	34	26.2	52.3
Graduate Degree	62	47.7	100.0
Total	130	100.0	

Table 11: Level of education of personnel operating recirculation facilities in the United States and Canada.

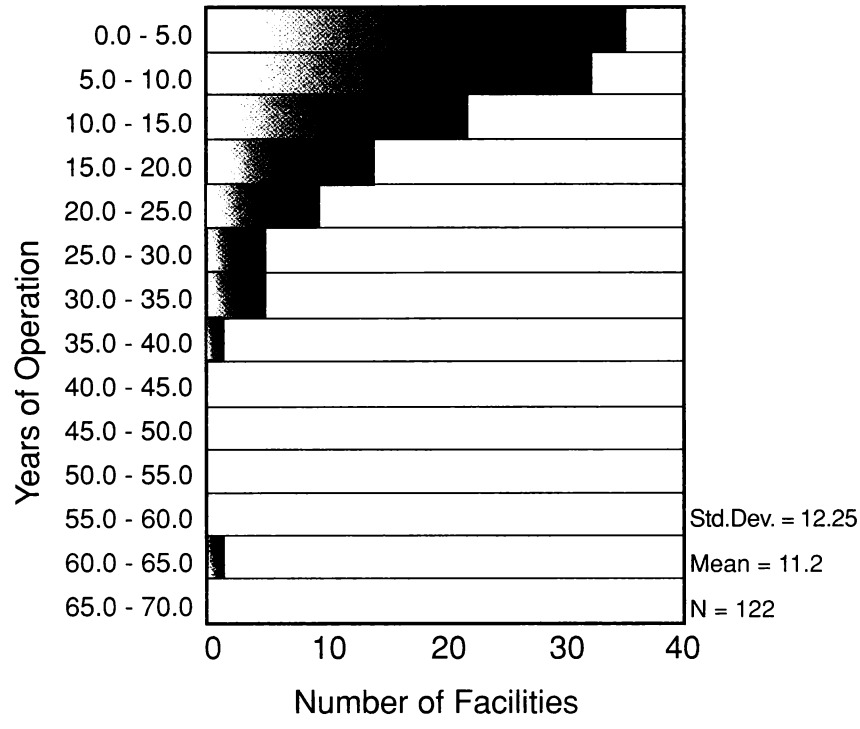


Figure 3: Age of facilities using recirculation technology to produce finfish in the United States and Canada.

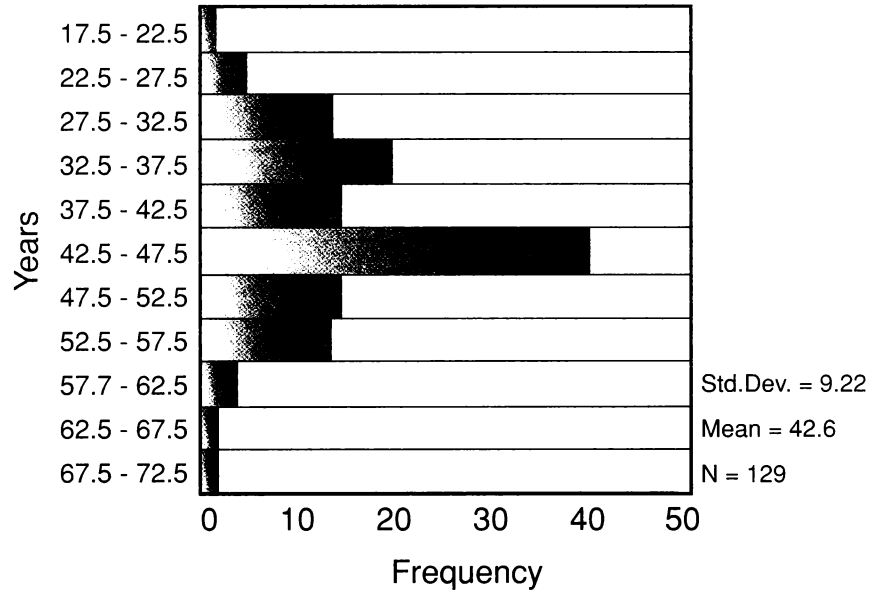


Figure 4: Age of personnel operating recirculation facilities in the United States and Canada.

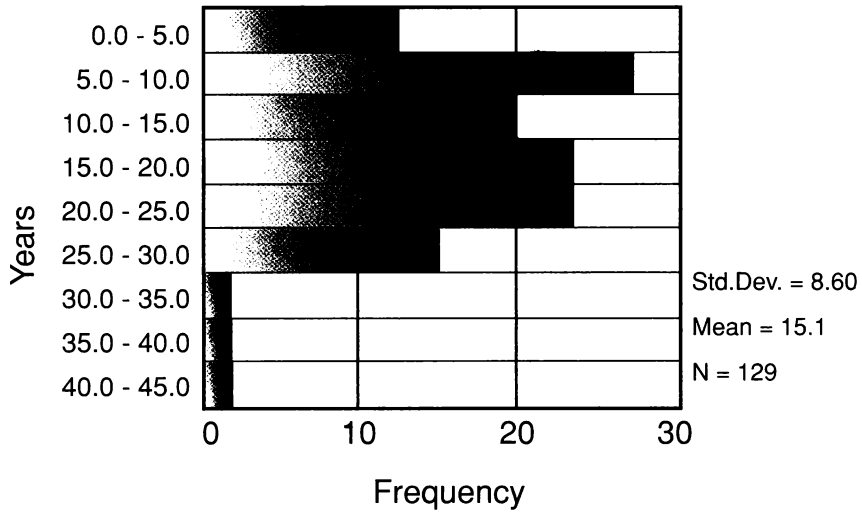


Figure 5: Years of work experience in aquaculture of personnel managing recirculation facilities in the United States and Canada.

DISCUSSION

Recirculating aquaculture systems have been under development in the United States and Canada for the past 30 years. As recently as 10 years ago, however, Masser et al. (1992) commented that "recirculating systems still have not proven to be an economical method of food fish culture". Data from this survey however, indicate that significant shifts have occurred in this sector of aquaculture during the past decade. Business enterprises, specifically facilities growing fish for the food fish market, are today the predominant users of recirculating technologies in the United States and Canada. Masser et al. (1992) also mentioned that "to date, most commercial systems have failed". Here, also, much has changed. Although business failure is common in aquaculture, the survey results show that the sector has expanded and diversified in size and purpose, and that many commercial operations have withstood the test of time. Our survey provides quantitative and qualitative information on many aspects of recirculation aquaculture including size of production, water sources used, and qualifications of personnel working in recirculation facilities. This background information is important for understanding the current status of this still-developing sector. Efforts continue in many areas around the United States and Canada to develop recirculating technologies as a means to grow fish efficiently. These technologies are expected to increase in

importance in future aquaculture production, driven largely by the increasing shortage of large quantities of suitable water needed for more traditional methods of finfish culture. Therefore, it seems appropriate that the current status of recirculating technology in United States and Canada be considered to provide a context for how best to develop this sector through the future. A thorough understanding of the constituents in a sector is a key requirement for successful oversight. Previous to this study, however, no information was available on the characteristics of the recirculation sector of aquaculture in the United States and Canada. For government agencies and policymakers to promote growth and to respond effectively to issues related to the recirculation sector of aquaculture, they must have an accurate account of the status of the sector. Likewise, up to this point, researchers have had little empirical information on the biological, physical and managerial characteristics of the recirculation sector of aquaculture, and were to that degree limited in providing research efforts that are applicable to the current situation. Armed with information collected in this study, educators and extension specialists should be better able to assist personnel within the recirculation sector with learning instruments and informational resources that are relevant to the sector's needs.

Our data show that the recirculating sector is diverse, both in terms of sizes of farms using recirculating technology and the types and life stages of fish grown. The current mix of small, medium and large farm enterprises suggests that this sector is quite early in its development. In most agri-businesses, because of the commercial realities of economy of scale, an industry eventually evolves to having few participants growing very large volumes of product. In the present group of respondents, there were five very large farms - possible indication that this sector is already evolving toward large volume production units. Government agencies, educators and extension specialists must plan their programming to account for the fact that annual production of only one of these large farms exceeds the total annual production of all of the farms grouped in the small-farm category (n=69).

One key finding of this study is that the recirculation sector of aquaculture is reliant on groundwater sources for its operation. Because of this, participants in this sector are vulnerable to changes in groundwater availability due either to changes in hydrological conditions or changes in policy or regulation regarding groundwater access and

consumption. Any restriction that is applied to groundwater use for aquaculture will impact this sector more severely than other finfish growing sectors. In addition, the findings suggest that research in support of recirculation technologies would fruitfully be directed towards solving key problems associated with the use of groundwater for fish culture. These problems include hazards associated with lethal gas levels, certain fish disease conditions which are more pronounced with the use of groundwater (i.e. nephrocalcinosis) and operational difficulties associated with some types of groundwater (Muir 1994). Our results also show that fully 40% of operations have no secondary water supply. Extension agents might discuss the importance of backup water supplies and explore technical alternatives with clients in their region.

The results of this study may have uncovered a key reason why some recirculation operations that are viable on paper do not succeed in reality. Theoretically, one of the major advantages of recirculation over flow-through culture of finfish is the reduced amount of water required to grow an equal volume of fish. The "cost" of accessing water in recirculation systems is supposedly lower. However, the "cost" of using a water source is a variable factor in the economics of these operations, and our survey indicates that within the recirculation sector, a broad range of water sources are used to grow fish. Pre-treatment water "costs" prior to applying the water to fish or eggs can include costs associated with dechlorination, purchase and installation of equipment to reduce incoming pathogen load, and construction of mixing chambers to adjust water chemistry. Likewise, the necessary post-treatment of a facility's effluent is often influenced by the source of its incoming water. Many economic models of recirculation technologies fail to account for the variety of incoming water sources used and the differences in costs associated with this choice of water source.

The recirculation sector has changed over time with respect to fish species grown. Only a few fish species are currently grown in large quantities using recirculation technology. The diversity of species that farmers have attempted to raise indicates that aquaculture enterprises are seeking and continue to experiment with alternative applications of the technology. At the same time, use of recirculation technology is an initially expensive fish culture endeavor, and therefore it must be acknowledged that market forces, not biological success, may drive the application of recirculating technology to new species.

Management personnel in the recirculating sector of aquaculture are highly educated. Because management capability is usually a combination of formal education and experience, it is quite promising for the development of this sector that the personnel managing these units have on average 10 years of related work experience. Although this sector is considered one of the newcomers to aquaculture production, it has a fairly solid human resource base with respect to skilled management personnel. Indeed, there is no other sector of agricultural enterprise with such a high frequency of higher levels of education at the management level. Government regulators, policy makers and extension agents should be aware of this level of education in order to interact effectively with this group. Vehicles of communication (e.g., workshops, extension bulletins) should provide information at a sophisticated level in order to be viewed as relevant and useful by this group of aquaculturists.

ACKNOWLEDGMENTS

This research was funded by the Department of Fisheries and Wildlife Sciences, College of Natural Resources, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

REFERENCES

Dillman, D. A. **1978**. Mail and Telephone Surveys: The Total Design Method. John Wiley & Sons, Inc. New York, NY, USA.

FAO Fisheries Department. Review of the State of World Aquaculture. FAO Fisheries Circular **1997**. No. 886 FIRI/C886 (Rev.1). FAO, Rome, Italy.

Losordo, T. M. Recirculating Aquaculture Production Systems: The Status and Future. *Aquaculture Magazine* **1998**, *24*[1], 38-45.

Losordo, T. M., Westerman, R. An Analysis of Biological, Economic, and Engineering Factors Affecting the Cost of Fish Production in Recirculating Aquaculture Systems. *Journal of the World Aquaculture Society* **1994**, *25*,103-203.

Masser, M. P., Rakocy, J., Losordo, T. M. Recirculating Aquaculture Tank Production Systems: Management of Recirculating Systems. SRAC Publication **1992**, No. 452, 1-12. Southern Regional Aquaculture Center, Mississippi State University, Starkville, MS, USA.

Michael, Don. West Virginia Department of Education, 1900 Kanawha Blvd, Charleston, WV, 25305, USA.

Muir, J. F. Water Reuse Systems in Aquaculture. *INFOFISH International* **1994**, *6*, 40-46.

Office of the Commissioner for Aquaculture Development (OCAD). Canadian Aquaculture Industry Profile **2001**. OCAD, Ottawa, Ontario, Canada.

Rosenthal, H. Environmental Issues and the Interaction of Aquaculture with Other Competing Resources Users. In Coldwater Aquaculture to the Year 2000. Proceedings of the Huntsman Marine Science Centre Symposium, St. Andrews, Canada, September 6-8 1995; Burt, M. D. B., Waddy, S.L. (Eds.) **1997**. Aquaculture Association Canada Special Publication 2, St. Andrews, NB, Canada.

United States Department of Agriculture. 1997 Census of Aquaculture (**1998**). Special Studies Part 3, AC97-SP-3, Washington, D.C., USA.

Estimating Metabolism of Fish in Aquacultural Production Systems

W. H. Neill[1]*, E. L. Oborny Jr. [2], S. R. Craig[3], M. D. Matlock[4], D. M. Gatlin III[1]

[1]* Department of Wildlife & Fisheries Sciences
Texas A&M University
College Station, TX 77843-2258 USA

[2] PBS&J
206 Wild Basin Road
Austin, TX 78746 USA

[3] VA-MD Regional College of Veterinary Medicine
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0442 USA

[4] Department of Agricultural Engineering
Texas A&M University
College Station, TX 77843-2121 USA

* Corresponding Author

ABSTRACT

Open-system respirometry offers a practical approach for measuring metabolic rates of fish cultured at high densities in uncovered raceways. Central to this methodology is analysis of a dynamic mass-balance on oxygen supply and demand. Here, we present a validated mass-balance equation, describe minimally disruptive procedures for estimating its parameters, and illustrate its use in estimating the oxygen-uptake rate of fish as a group, in real time and under actual production conditions.

INTRODUCTION

Oxygen respirometry is the dominant technique for estimating aerobic metabolism of fish and other water-breathing animals. In effect, the rate of oxygen-uptake by a fish in a closed or semi-closed chamber is presumed to be equivalent to the rate of oxygen disappearance from the water contained in or flowing through the chamber. The equivalency may or may not be adjusted for disappearance or appearance of oxygen in a "blank" control chamber, attributable to microbial activity. Static respirometers have only the water movement necessary to assure mixing

and adequate irrigation of the oxygen electrode; active respirometers are intended for measuring oxygen-uptake rate in fish forced to swim at constant speed against a water current. Cech (1990) has provided a thorough review of conventional respirometry. Springer and Neill (1988) have described the development of computer-automated respirometry.

Respirometry as described above, is more suited to the research laboratory than the fish farm. The object of study generally is metabolism of a fasted, isolated fish, confined in a small glass or plastic chamber under controlled conditions of lighting (typically dim or dark) and temperature. If the fish is forced to swim at maximum sustainable speed, "active" metabolism is estimated; otherwise, "standard" or "routine" metabolism is observed. Some who have made such measurements (e.g., Neill and Bryan 1991) have expressed concern about their applicability to more normal situations. Such concern motivated us to consider a more direct approach to respirometry in one "real-world" situation—intensive aquaculture in raceways — a situation in which the strong metabolic signal from a very concentrated fish biomass overwhelms the noise that otherwise might defeat the approach.

METHODS AND MATERIALS

Open-system respirometry

Oxygen uptake rates of fish in aquacultural production systems can be estimated from continuously (or intermittently) recorded oxygen concentration data, by solving for M in the equation

1) $(dOc/dt)*C = (Oi-Oc)*Q + (Os-Oc)*K + M + BCOD$

where:

Oc = O_2 concentration in raceway (or the system compartment containing the fish) and effluent from raceway, mg/L;

Oi = O_2 concentration in influent to raceway, mg/L;

Os = O_2 concentration in raceway at gas saturation, mg/L, where the gas is air or oxygen-enriched air;

C = raceway volume, L;

Q = water exchange rate, L/t (t = time);

K = reaeration rate, L (water aerated)/t;

M = rate of oxygen removal attributable to metabolism of fish, mg O_2/t;

BCOD = rate of oxygen removal (rarely, resupply) attributable to other biological and chemical oxygen "demand" processes, mg O_2/t.

In effect, this equation states that the time-rate of change in dissolved-oxygen concentration of a well-mixed production tank with volume C is the resultant of oxygen supply and use. The first two terms on the right side of the equation normally are positive; they represent net rates of oxygen-concentration change attributable to water exchange and reaeration, respectively. The demand terms, BCOD and M, normally are negative (although, rarely, photosynthesis can cause BCOD to be positive).

All variables and parameters in this mass-balance equation can be measured easily and directly except for K and BCOD (Figure 1). The reaeration rate K is a measure of how effectively the raceway is resupplied with oxygen via aeration or injection of oxygen (in closed-system respirometry, K is zero). Estimation of K requires that the system be perturbed, in that Oc must be displaced from its steady-state value, Oc' (or vice versa); then, K can be computed from the rate at which Oc approaches the new Oc'. The perturbation must be accomplished without changing the system dynamics. Two methods have been utilized to displace Oc from its steady-state value: 1) temporarily infusing oxygen or nitrogen, to displace Oc from Oc'; or 2) zeroing M, by

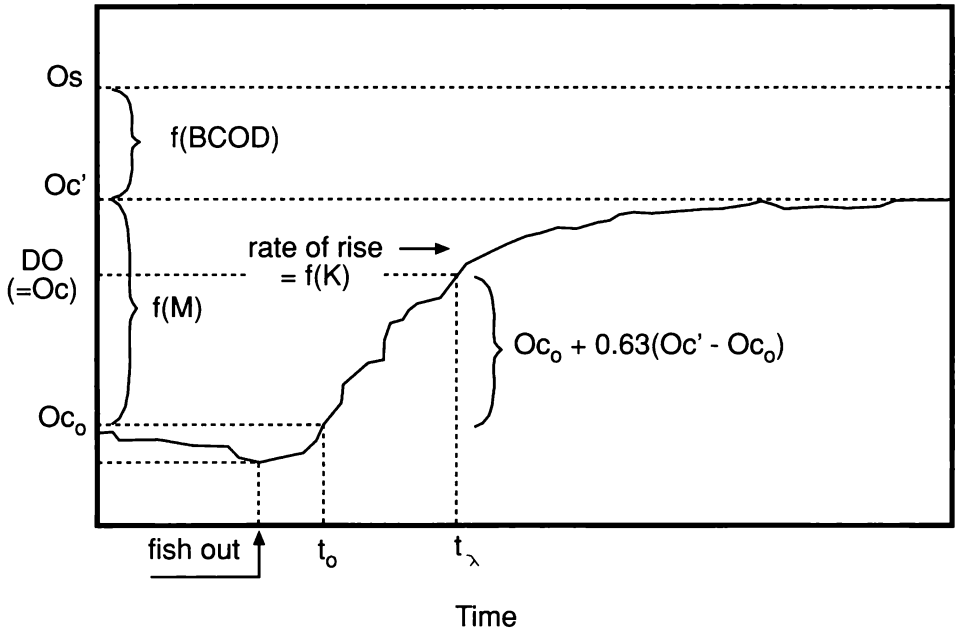


Figure 1. Pattern of change in dissolved oxygen concentration (DO) in an uncovered raceway, before and after removal of fish. See text and Appendix for interpretation.

removing the fish from the system, to displace Oc' from Oc. The first method avoids the work and disruption of moving the fish; in addition, any stirring of the water by the swimming activity of the fish, which may be an important component of K, remains in effect. However, the second method allows what normally should be better estimation of BCOD (see below). It also affords the opportunity to measure fish sizes and total biomass. Under production conditions, the removal of fish from the raceway would be impractical, except when the respirometry trial coincides with a planned fish transfer or harvest.

In any case (whether or not M = 0), at steady state

2) $M + BCOD = - (Oi - Oc')*Q - (Os - Oc')*K.$

Then, for the transient state,

3) $(dOc/dt)*C$
$= (Oi - Oc)*Q + (Os - Oc)*K - (Oi - Oc')*Q - (Os -Oc')*K$
$= (Oc'- Oc)*(K + Q).$

Thus, Oc approaches Oc' as an exponential decay process, with the rate coefficient equal (K + Q)/C; so, K can be estimated by finding the 63% time constant for the response in Oc (see Appendix), taking its inverse, multiplying the result by C, and finally subtracting Q.

Only the BCOD in the production tank itself is relevant since other BCOD, such as that in an external biofilter or other plumbing, will manifest itself as an effect on Oi. If most of the relevant BCOD is that associated with dissolved or suspended materials, BCOD can be estimated by measuring rate of oxygen-concentration change (normally, a decrease), dObcod/dt, in $mgO_2/(L*t)$, in a water sample contained in a "light" bottle incubated at mid-depth in the production tank:

4) $BCOD = dObcod/dt*C.$

In many production systems, however, a large fraction of relevant BCOD may be associated with surfaces. In that case, a better estimate of BCOD will be obtained by solving equation 2 with M set to zero—i.e., with the fish removed from the tank:

5) $BCOD = - (Oi - Oc')*Q - (Os - Oc')*K.$

Now, with numeric estimates both for K and BCOD in hand, the parent equation (1) can be solved for M:

6) $M = (dOc/dt)*C - (Oi - Oc)*Q - (Os - Oc)*K - BCOD.$

The aquacultural production systems envisioned in developing this analytical approach, were well-mixed, uncovered tanks or raceways with either once through flow or recirculation of water from an external biofilter. In the case of a tank with internal biofilter or a system with negligible differences between Oi and Oc, one simply deletes the water exchange term (but, in the latter case, not Q in the computation of K!) and, for recirculating systems, excludes from C the volume of water in any external biofilter and other plumbing. In principle, there is no reason our methodology could not be applied to earthen ponds, provided they are sufficiently well-mixed to be without marked oxygen gradients. Any photosynthetic production of oxygen or plant respiration would show up in the BCOD term and could be expected to impart a diel cycle on Oc, independent of M.

RESULTS

A rectangular fiberglass raceway at Texas A&M University System's Aquacultural Research and Teaching Facility (Burleson Co., TX, USA) contained approximately 350 500-g red drum (*Sciaenops ocellatus*) in 7,000 L of 3 ppt artificial seawater. These fish were removed from the raceway and weighed, for a total biomass of 175.05 kg. Just before the fish were disturbed, DO was 3.4 mg O_2/L = Oc and declining at 0.05 mg O_2/L per minute [dOc/dt = -0.05 mg O_2/(L*min) = -3.0 mg O_2/(L*h)]; after the fish were removed, DO rose from 3.0 mg O_2/L to a new steady state of 5.3 mg O_2/L = Oc'. Time for 63% of the change (from 3.0 to 4.5 mg O_2/L) was 42 minutes, or 0.70 hours; thus, K = (1/0.7)*7,000 = 10,000 Lh^{-1}. (In this case, the internal biofilter's volume is included in C for the system, and Q is taken as zero.) Water temperature was approximately 27°C; so, Os was taken to be 7.7 mg O_2/L.

7) BCOD = - (Os - Oc')*K
= - (7.7 - 5.3)*10,000
= -24,000 mg O_2/h.

For Oc at 3.4 mg O_2/L and declining at 0.05 mg O_2/(L*min) = 3.0 mg O_2/(L*h),

8) M = (dOc/dt)*C - (Os - Oc)*K - BCOD
= (-3.0)*7,000 - (7.7 - 3.4)*10,000 - (-24,000)
= - 21,000 - 43,000 + 24,000
= - 40,000 mg O_2/h.

Thus, at the moment of interest, metabolic rate of the fish per gram body weight was 40,000/175,050 = 0.23 mg O_2/(g*h). Is this value right or wrong? It can only be stated that this number is consistent with results from closed-system respirometry (Forsberg and Neill 1998). Also, validation work by Oborny (1993) gives us further confidence in the methodology.

DISCUSSION

Oborny (1993) has validated the physics, the biology, and the practicality of open-system respirometry as described here. In addition, he showed that the approach can be extended to accommodate oxygen-enriched systems, simply by setting Os to its supersaturated value. Following is a synopsis the validation studies conducted by Oborny (1993).

Open-system respirometry was physically validated by simulating fish metabolism via constant inflow of oxygen-deficient water into a well-stirred aquarium open to the atmosphere. These trials involved oxygenation of the aquarium both with air and pure oxygen. Calculated metabolism compared very favorably with known rates of oxygen dilution, for both regimes of oxygenation: $r^2 = 0.98$ for air and 0.92 for pure oxygen.

To validate open-system respirometry in a biological sense, Oborny (1993) compared whole-body energy changes in unfed juvenile red drum, measured via proximate analysis and bomb calorimetry, with those estimated from apparent oxygen uptake via open-system respirometry. For three independent trials, the energy loss measured by respirometry was 95.8, 97.7, and 102.1% of that measured by direct calorimetry.

Finally, Oborny (1993) put open-system respirometry to a practical test in large-scale, intensive raceways at a commercial red drum production facility. The experiment compared the proportion of apparent oxygen consumption to the proportion of fish biomass remaining, as fish were harvested from each of two 113,550 L systems. In one system, 80% of the fish consumed 71% of the oxygen consumed by all the fish (on the previous day). The second system yielded 25% oxygen consumption for 33% of the fish biomass. The metabolic rates of the 170-200 g fish in these large systems, at biomass densities up to 0.075 kg/L, ranged from 0.45 to 0.66 mg O_2/ (g*h).

ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the Texas Sea Grant College Program.

REFERENCES

Cech, J. J., Jr. Respirometry. In Methods for Fish Biology. Schreck, C.B. Moyle, P.B. (Eds.) **1990**. Chapter 10, pages 335-362. American Fisheries Society, Bethesda, MD, USA.

Forsberg, J. A., Neill, W. H. Saline Groundwater as an Aquacultural Medium: Physiological Studies on the Red Drum, *Sciaenops ocellatus*. *Environmental Biology of Fishes* **1998**. *49*, 119-128.

Oborny, E. L., Jr. **1993**. Open-System Respirometry in Intensive Aquaculture: Model Validation and Application to Red Drum (*Sciaenops ocellatus*). M.S. thesis, Texas A&M University, College Station, TX, USA.

Neill, W. H., Bryan, J. D. **1991**. Responses of Fish to Temperature and Oxygen, and Response Integration Through Metabolic Scope. In Aquaculture and Water Quality: Advances in World Aquaculture, Vol. 3. Brune, D. E., Tomasso, J. R. (Eds.), p. 30-57. The World Aquaculture Society, Baton Rouge, LA, USA.

Springer, T. A., Neill, W. H. Automated Determination of Critical Oxygen Concentration for Routinely Active Fish. *Environmental Biology of Fishes* **1988**. *23*, 233-240.

APPENDIX

More on estimating K and Oc'

- K can be estimated by measuring time for raceway Oc to change from any particular value to a value 63% of the way toward the steady-state value (Figure 1). This time interval, $t_{lambda} - t_0$, is *lambda*, the time constant for the system. For negative exponential processes, *lambda* = 1/k, where k is the exponential rate constant and has units of $(time)^{-1}$.

$$k = (K + Q)/C; \quad \text{(A1)}$$

so,

$$K = k*C - Q = (1/lambda)*C - Q. \quad \text{(A2)}$$

- Alternatively, k can be estimated as -1*slope of the linear regression of $\ln[(Oc - Oc')/(Oc_0 - Oc')]$ on t (which is the more conservative method for estimating negative-exponential rate constants). Then, K can be found, as before, by solving equation A2.

- Of course, both approaches assume that Oc' (and, thus, BCOD and Os), C, Q, and K are representative and constant , at least during the interval (t_0, t_{lambda}). Experience indicates that for most recirculating aquaculture systems, *lambda* will be on the order of 0.5 hour. So, measurement of Oc at intervals of 10 minutes for one hour beginning at to should be sufficient for estimating k via either method.

- Oc', in practice, typically would be estimated after the Oc observations that are collected to estimate K. If the reaeration process is a negative exponential (and it almost certainly is), then Oc should have advanced 95% of the way from Oc_0 to Oc' in 3 *lambda* or in about 1.5 hours, for most systems. To be safe, one should wait 2.5 hours to measure Oc as an estimate of Oc', but not longer than 4 hours, because BCOD may have begun to change after the nutrient sources have been absent from the system for this long.

- Summary of data needs for estimation of K and Oc': 1) Value of Oc soon after animals removed from the system (but after the system is otherwise restored to normal operating conditions, water level, air lifts, etc.); this fixes Oc_0. 2) Values of Oc every 5 to 15 minutes thereafter for at least 1 hour. 3) Value of Oc 2.5 to 4 hours after, to estimate Oc'.

Evaluation of Dissolved Chitosan for Suspended Solids Removal

S. Tsukuda, J. Davidson, E. Adkins, S. Summerfelt*

The Conservation Fund Freshwater Institute
P.O. Box 1889
Shepherdstown, WV 25443 USA
E-mail: s.summerfelt@freshwaterinstitute.org

* Corresponding Author

ABSTRACT

In a preliminary study conducted at The Conservation Fund Freshwater Institute (Shepherdstown, WV, USA), dissolved chitosan was added to a recirculating system to determine if the chitosan would coagulate particulate matter and consequently increase solids removal. The recirculating water became visibly clearer and the culture tank total suspended solids (TSS) concentration dropped from 10.7 to 2.9 mg/L within 2 hours after dosing had been initiated. However, fish showed symptoms of distress and the chitosan treatment was discontinued. In subsequent studies conducted to determine the particle capture mechanism associated with chitosan addition, effluent treated with dissolved chitosan was not returned to the system. The results of two jar test studies indicated that dissolved chitosan did not enhance particle capture by settling or by microscreen filtration when mixed with a fish culture system effluent containing *10 mg/L of TSS. However, these jar tests indicated that an additional 44% of TSS could be removed from the water that had already passed through a microscreen filter if this water was treated by a mixing and settling step, even without addition of dissolved chitosan. Additional studies using small-scale fluidized-sand biofilters indicated that the reduction in TSS observed in our initial experiment was due to TSS capture in the fluidized sand biofilter. TSS concentrations were reduced from 5.1-7.4 mg/L at the biofilter inlet to 1.7-2.2 mg/L at the biofilter outlet. Thus, adding dissolved chitosan to water flowing into a fluidized-sand biofilter turned the biofilter into a novel type of upflow 'sludge blanket clarifier,' which appears to be both non-plugging and relatively simple to operate. In addition, dissolved chitosan did not change nitrification occurring within the fluidized-sand biofilter. Therefore, adding a coagulant (such as dissolved chitosan or a

non-toxic polymer) to the flow entering a fluidized sand biofilter has the potential to create a unit process that reduces TSS while simultaneously treating dissolved wastes.

INTRODUCTION

Organic suspended solids encountered in aquaculture systems will contain phosphorus, can contain undesirable organisms, and may cause gill irritation in salmonids (Noble and Summerfelt 1996). Organic matter can also degrade and release ammonia and create a biochemical oxygen demand. Suspended solids must be removed from recirculating aquaculture systems to improve water quality. In addition, suspended solids must also be removed from their effluents in order to meet state and federal effluent discharge limits. Sedimentation and microscreen filtration are the primary mechanisms used to remove particulate matter from coldwater recirculating systems and their effluents. However, sedimentation and microscreen filtration units typically do not remove particles much smaller than about 75 mm (Timmons et al. 2002), which might not be adequate because particles that can contribute to gill irritation and mortality may be in the 5-10 mm range (Chapman et al. 1987). Other options that can be used to increase the removal of fine particles include foam fractionation (Weeks et al. 1992), ozonation (Summerfelt et al. 1997), and possibly the addition of flocculation aids such as ferric chloride, alum, and/or polymers (Ebeling et al. In Review).

Chitosan is an organic, cationic polymer commonly derived from chitin extracted from the exoskeletons of crustacean for use in a variety of commercial applications. Chitosan has been touted as a non-toxic coagulant that is widely applied in wastewater and agricultural applications and that is also being studied for uses in human medicine (Sandford 1989, Elson 1996). Dissolved chitosan has been used at doses of 0.15-1.0 mg/L as a coagulant or coagulant aid to increase solids removal in various surface water treatment applications (Vaidya and Bulusu 1984, Kawamura 1991) and in wastewater treatment and food processing applications (Bough 1976, Wu et al. 1978). Feeding, injecting, and bathing rainbow trout (*Oncorhynchus mykiss*) in chitosan solutions has been shown to be a non-toxic and effective immunostimulant (Anderson and Siwicki 1994, Siwicki et al. 1994).

Chitosan has also been reported to be non-toxic when ingested by fish (Kono et al. 1987). Acidified chitosan that had been dissolved in malic acid was reported to be non-toxic to fathead minnows (*Pimephales promelas*) in a Technical Data Sheet (Sea Klear Chitosan Toxicity Data 11/8/96) provided by Vanson (Redmond, WA, USA). Based on our literature search, we found no indication that dissolved chitosan would be toxic to fish.

The purpose of this research was to determine if low doses of dissolved chitosan would produce coagulation and flocculation of fine particulate organic matter and thus increase solids removal within recirculating aquaculture systems or from their effluent.

MATERIALS AND METHODS

Dissolved chitosan stock solution

A 1% chitosan (10,000 mg chitosan/L) stock solution was used in the study. For reasons of material availability, this solution was prepared by one of two methods: (1) 10 g chitosan dissolved in 100 mL of 10% acetic acid and 900 mL distilled water (2) 10 g chitosan dissolved in 10 mL glacial acetic acid and 990 mL distilled water. For the jar tests, further dilutions of the stock solution were prepared to produce uniform 10 mL doses into the 2 L jars. For example, for a 0.1 mg/L final jar concentration of chitosan, the chitosan stock was diluted to produce a 20 mg/L chitosan dosing solution.

Chitosan dosed into a coldwater recirculating system

In a preliminary study conducted at the Conservation Fund Freshwater Institute, dissolved chitosan was added to a recirculating system (Figure 1) to determine if the chitosan would coagulate solids and consequently increase solids removal. The recirculating system (Figure 1) has been described elsewhere (Heinen et al. 1996a). Dissolved chitosan was added to create a concentration of 1 mg/L in the recirculating flow entering the fish culture tanks. The concentration of TSS in the water exiting the culture tank was measured 2 hours after chitosan addition had begun. The experiment was terminated at this point due to chitosan toxicity problems that had become apparent.

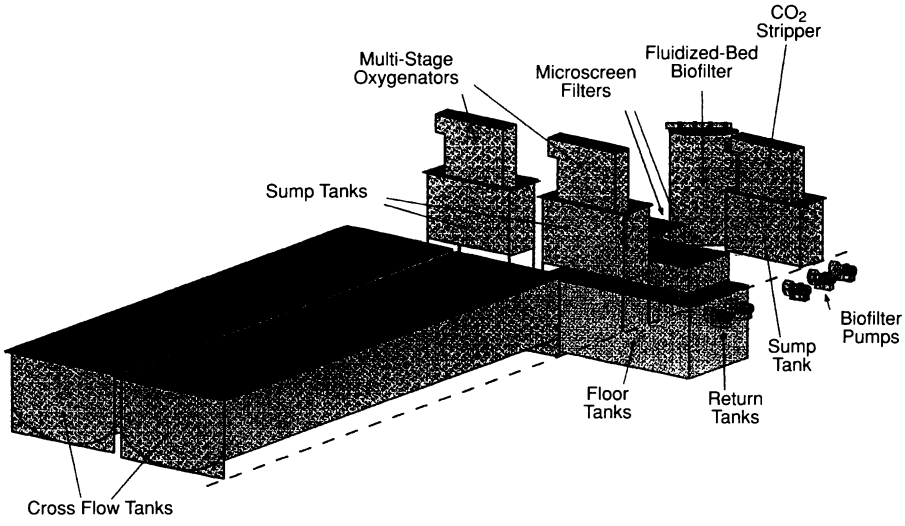


Figure 1: Illustration of the recirculating culture system used in this study (Heinen et al., 1996a)

Jar test studies

The effects of dissolved chitosan on TSS coagulation and flocculation were evaluated using jar test methods. Two series of jar tests were run using water samples that were collected either before or after an 80 mm Hydrotech (Vellinge, Sweden) microscreen filter unit. Both jar tests utilized square cross-sectioned Wagner floc jars (11.5 x 11.5 x 21 cm) with a sampling tap positioned 5 cm from the bottom of the jar. Samples were stirred with a Phipps and Bird six-paddle stirrer (Model 7790-400, Richmond, VA, USA) with a rectangular paddle blade (76 cm x 25 cm).

For the first jar test series, each of the six Wagner floc jars received 2 L of water collected following microscreen filtration. Next, the jars were dosed with the appropriate 10 mL dose to produce 0.025, 0.050, 0.10, 0.20 and 0.40 mg/L chitosan. The jars were then flash mixed at 100 rpm for 1 minute, floc mixed at 30 rpm for 20 minutes and then allowed to settle for 30 minutes. Finally, a 1 L sample was collected through the sampling port from each jar and these samples were analyzed for TSS, color and turbidity using standard methods (APHA 1989). These analyses were also performed on a 1 L unmixed control sample.

The second jar test series examined effluent leaving the fish tanks prior to microscreen filtration. Jars were dosed with 0.0, 0.1 or 0.4 mg/L chitosan. The data from the two replications were averaged. Following

the 1 minute flash mix and 20 minute floc mix, the full 2 L of treated effluent was collected from each jar. The treated effluent was passed through successively smaller nylon net filters and finally through a standard TSS filter paper to capture the remaining solids. Millipore Nylon Net Filters (Bedford, MA, USA) sized 120, 80, 41, 20 and 11 mm and a Gelman Glass Fiber Filter (Pittsburgh, PA, USA) rated nominally at 1 mm were used. The mass of solids on each of the screens was measured using the standard method for TSS analysis (APHA 1989). The screen filters were used to determine if particle size distribution was altered by chitosan addition.

Sweep floc removal of TSS within pilot-scale fluidized-sand biofilters

Three pilot-scale biofilters were used in this study. Each column was 16.2 cm in diameter and 2.5 m tall. Immediately before each trial began, 9 L of actively nitrifying sand was taken from the main system biofilter and was transferred into each of the test columns. After being filled with sand, the pilot-scale biofilters were fluidized and allowed to stabilize for 60 hours prior to dosing. Each of the columns received tank effluent after it had passed through the microscreen filter. Dosing began at 9:00 a.m. and continued for 48 hours. Cole-Parmer (Chicago, IL, USA) peristaltic pumps were used to supply the pilot-scale biofilters with water from the recirculating system. Chitosan doses of between 0.44 and 0.55 mg/L were applied. Columns dosed solely with acetic acid had concentrations between 0.44-0.45 μL acetic acid per liter effluent, which is a concentration equivalent to the acetic acid concentrations in the columns dosed with dissolved chitosan solution. The fluidized bed heights were measured at time 0, 2, 4, 6, 24, 26, 28, and 30 hours. Other biofilter influent water conditions were as follows: average flow = 6.9 L/min, temperature = 15.1, pH = 7.6, alkalinity = 240 mg/L.

Water quality parameters were monitored to determine effects of chitosan dosing on biofilter performance. Equipment used included a YSI Model 58 dissolved oxygen meter (Yellow Springs, CO, USA) and Fisher Scientific Accumet pH meter 915 (Pittsburgh, PA, USA). A DR/2000 spectrophotometer utilizing the Nessler method and Diazotization method were used to test total ammonia nitrogen and nitrite nitrogen, respectively, using methods developed by Hach Company (Loveland, CO, USA). Sampling was conducted at t = 0, 2, 4, 6, 24, 28, 30, and 48 hours. Samples for TSS were collected at t = 0, 1, 3, 6, 24, and 30 hours.

RESULTS AND DISCUSSION

Chitosan dosed into a coldwater recirculating system

In the preliminary study, where dissolved chitosan was added to a recirculating system (Figure 1), the recirculating water had become visibly clearer within 2 hrs of initiation of chitosan addition, and the culture tank TSS levels had dropped from 10.7 to 2.9 mg/L. However, fish began to show symptoms of distress after 2 hrs of exposure to chitosan, so the treatment was discontinued. Mortality of 4.6% was observed over the next 24 hours. Nitrification was not affected by the short-term dose of dissolved chitosan. The toxicity of dissolved chitosan to rainbow trout was a surprise based on the extensive literature review that had been conducted. Following this incident, detailed toxicity trials and histological examinations on rainbow trout indicated that dissolved chitosan concentrations as low as 0.019-0.038 mg/L caused lifting of lamellar epithelium, hypertrophy, and hyperplasia of lamellar epithelial cells while concentrations of 0.075 mg/L caused mortality after 24 hours (Bullock et al. 2000).

The preliminary study did indicate that dissolved chitosan improved TSS removal from the recirculating flow. However, additional tests were required to determine exactly how chitosan improved particle capture. Did dissolved chitosan coagulate particles and increase the rate that they settle or are they removed by microscreen filtration? Or, did chitosan cause particles to stick to the biosolids found in the recirculating system's fluidized-sand biofilter? In either case, the application of dissolved chitosan had now become of interest only from an effluent treatment stand-point. Therefore, in our subsequent studies, we applied dissolved chitosan to water that had been removed from the recirculating system to avoid further exposing fish to chitosan.

Jar test studies

Jar test results are shown in Table 1. A one-way analysis of variance was performed on the data. The TSS, color, or turbidity measurements were not found to be significantly different among levels of chitosan addition. We thought that chitosan may have inhibited particle settling by attaching to the particles and making them nearly neutrally buoyant. Our hypothesis was based on a report by Vaidya and Bulusu (1984) that

dissolved chitosan added to turbid water created a "floc [that] was light and settled slowly."

Of note, data from the zero chitosan jar tests (i.e., at the 0.0 mg/L chitosan dose in Table 1) indicated that an additional 44% of the TSS could be removed from the filtered water discharged from the microscreen filter if this water was then treated by a mixing and settling step - even without chitosan addition. While microscreen filtration is important to quickly remove cBOD and ammonia contained in the solids, this research indicates greater particle capture could be achieved by installing a mixing and settling step after the microscreen filter.

After the first jar test studies, we thought that the chitosan and mixing steps might be creating a larger floc that was not settling. To verify our hypothesis, in a second jar test study the full 2 L of water was removed from the jars after the 20 minute flocculation-mixing step was completed. This water was then passed through successively smaller filter screens. The water sample was passed through one screen at a time, starting with the largest, and then through screens with progressively smaller openings. The focus of this series of tests was to determine if chitosan addition changed the particle removal across the different sized screens. If chitosan addition increased the particle removal across the screens with the largest openings, then chitosan addition could be used to enhance solids removal efficiency using microscreen filtration.

The results from passing the flocculated water samples through progressively smaller screen openings indicated that the screen with the largest openings (i.e., 120 mm) captured nearly 80% of TSS in the flocculated water sample (Figure 2). TSS capture did not differ significantly among levels of chitosan addition, i.e., 0.00, 0.10, and 0.40 mg/L of chitosan dose (Figure 2). Therefore, there was no indication that chitosan addition produced a larger floc, which would improve particulate capture across a microscreen filter. Interestingly, these results also suggest that pre-treating water before it enters a microscreen filter with a 20 minute flocculation step could increase the TSS capture efficiency across a 120 μm sieve panel to approximately 80%. In contrast, without a 20 minute flocculation pretreatment step, the microscreen filters that contained 80 μm sieve panels only removed 50-60% of the TSS loading within the recirculating system (Heinen et al. 1996b).

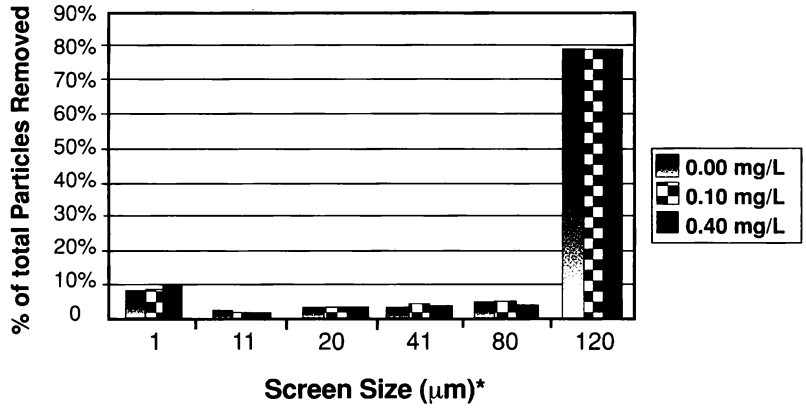


Figure 2: Percentage of total particles removed by each screen (by mass) at the three dose levels of chitosan applied to fish tank effluent.

Sweep floc removal of TSS within pilot-scale fluidized-sand biofilters

After concluding the jar test studies, the use of dissolved chitosan would probably not have been deemed viable for commercial scale aquaculture. However, we observed a large increase in water clarity and reduced TSS concentration following only 2 hours of chitosan addition to the recirculating system. After ruling out the possibility that chitosan increased TSS removal across the drum filter, it was determined that TSS capture within the fluidized-sand biofilter was the most likely explanation of the solids removal that occurred in the preliminary study.

Recirculating system water was pumped through three replicated pilot-scale fluidized-sand biofilter columns to determine if either dissolved chitosan or acetic acid (at a concentration equivalent that in the dissolved chitosan dose) increased TSS capture across the biofilter columns, changed the bed expansion and growth within biofilter columns, or inhibited nitrification activity.

While all columns removed TSS (Tables 2 and 3), addition of dissolved chitosan caused the fluidized-sand biofilter to remove 2-3 times more TSS than the columns dosed with the acetic acid and the columns that had no acetic acid or chitosan addition. The columns dosed with 0.44-0.55 mg/L of dissolved chitosan produced effluent TSS concentrations that were 1.7-2.2 mg/L (Table 2), which indicates the presence of an effective TSS capture mechanism within the expanded bed. In addition, the dissolved chitosan doses applied did not negatively affect the total ammonia nitrogen (TAN), nitrite nitrogen, dissolved oxygen, or pH of the water discharged from the biofilter columns (Tables 4-7).

Parameter	Before jar test	Following jar test at each dose of dissolved chitosan (mg/L) 0.000	0.025	0.050	0.10	0.20	0.40
TSS (mg/L)	9.8±0.7	5.5±0.2	5.4±0.3	5.4±0.3	6.1±0.3	6.1±0.2	6.3±0.3
True color (Pt-Co)	18±1	17±1	17±1	17±1	17±1	16±1	16±1
Turbidity (NTU)	3.3±0.3	2.6±0.1	2.5±0.1	2.4±0.1	2.6±0.1	2.6±0.1	2.6±0.1

Table 1: TSS, color, and turbidity levels (Mean ± SE) of water samples taken from the recirculating system (after the microscreen filter) both before and after the samples had been jar tested at each dissolved chitosan dose.

		Effuent of fluidized-sand biofilter		
	Influent	No dose	Acetic acid only	Dissolved chitosan
Trial 1	7.4 ± 0.3	5.0 ± 0.4	not tested	2.2 ± 0.2
Trial 2	5.1 ± 0.2	4.0 ± 0.1	4.1 ± 0.2	2.2 ± 0.3
Trial 3	5.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	1.7 ± 0.2

Table 2: Mean (± SE) fluidized-sand biofilter influent and effluent TSS concentrations (mg/L) measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

	No dose	Acetic acid only	Dissolved chitosan
Trial 1	33 ± 7	not tested	70 ± 7
Trial 2	20 ± 4	22 ± 3	62 ± 5
Trial 3	44 ± 3	44 ± 3	72 ± 5

Table 3: Mean TSS capture efficiency (% ± SE) across the fluidized-sand biofilter columns measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effuent of fluidized-sand biofilter		
	Influent	No dose	Acetic acid only	Dissolved chitosan
Trial 1	10.6 ± 0.06	7.0 ± 0.08	not tested	6.9 ± 0.08
Trial 2	10.6 ± 0.04	7.7 ± 0.04	7.6 ± 0.05	7.6 ± 0.05
Trial 3	10.3 ± 0.06	7.2 ± 0.09	6.9 ± 0.05	6.8 ± 0.04

Table 4: Mean (% ± SE) dissolved oxygen across the fluidized-sand biofilter columns measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	7.4 ± 0.3	5.0 ± 0.4	not tested	2.2 ± 0.2
Trial 2	5.1 ± 0.2	4.0 ± 0.1	4.1 ± 0.2	2.2 ± 0.3
Trial 3	5.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	1.7 ± 0.2

Table 5: Mean (% ± SE) fluidized-sand biofilter influent and effluent pH measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	0.42 ± 0.01	0.04 ± 0.00	not tested	0.04 ± 0.01
Trial 2	0.36 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
Trial 3	0.39 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

Table 6: Mean (% ± SE) fluidized-sand biofilter influent and effluent TAN measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

		Effluent of fluidized-sand biofilter		
	Influent	*No dose*	*Acetic acid only*	*Dissolved chitosan*
Trial 1	0.020 ± 0.000	0.005 ± 0.000	not tested	0.003 ± 0.000
Trial 2	0.021 ± 0.001	0.006 ± 0.000	0.005 ± 0.000	0.003 ± 0.000
Trial 3	0.027 ± 0.001	0.007 ± 0.001	0.005 ± 0.000	0.003 ± 0.000

Table 7: Mean (% ± SE) fluidized-sand biofilter influent and effluent nitrate concentrations (mg/L) measured from 1 to 48 hours after the initiation of chitosan or acetic acid dosing.

The fluidized-sand biofilter bed exposed to the 0.44-0.55 mg/L of dissolved chitosan feed initially contracted (Figure 3). However, because of the higher TSS capture rate within the chitosan-dosed column, the fluidized bed depth in the chitosan-dosed column grew faster and eventually equaled the depth of the other two treatments at the end of the experimental period (Figure 3). It remains to be investigated what will happen to the solids over a longer dosing period and how those solids will be managed.

The dissolved chitosan appears to have adsorbed to particles in the fluidized-sand biofilter, which created a novel type of upflow 'sludge blanket clarifier' utilizing the biosolids blanket contained in the fluidized bed. With dissolved chitosan creating particle coagulation, the fluidized-

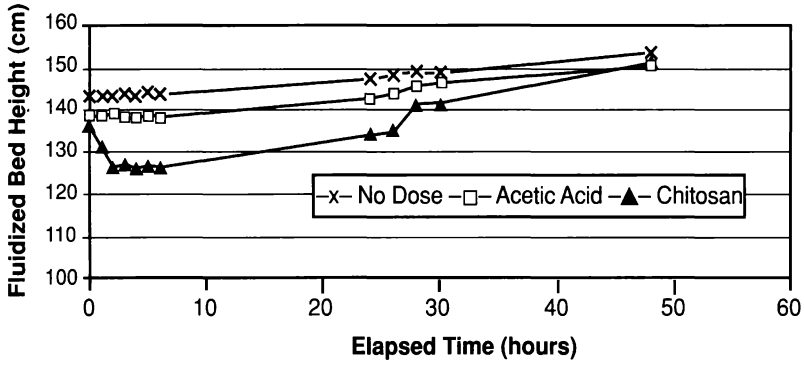


Figure 3: Expanded bed height measured within the pilot-scale fluidized-sand biofilter columns after chitosan or acetic acid dosing had begun, i.e., at time = 0.0 hours.

sand biofilter performed as an upflow 'sludge blanket clarifier' somewhat similar to a solids contact unit that recirculates settled solids, as described by Culp et al. (1978). This preliminary study indicates that fluidized-sand biofilters could be used as a type of upflow 'sludge blanket clarifier' to remove both dissolved and particulate wastes from the effluent of a recirculating aquaculture system. Use of a fluidized-sand biofilter in this application would have advantages because the expanded bed would be non-plugging and the unit would be relatively simple to operate because it would never require backwashing. Biosolids captured in the expanded bed would be simply siphoned off of the top of the bed in a manner similar to that which is used to remove bed growth in commercial fluidized-sand biofilters (Summerfelt et al. 2001).

CONCLUSIONS AND RECOMMENDATIONS

Chitosan was observed to be acutely toxic to rainbow trout at low levels (<1 mg/L). Therefore, dissolved chitosan should not be added to aquaculture systems containing rainbow trout. It is unknown whether dissolved chitosan is as toxic to other aquatic species. Although dissolved chitosan addition was not effective at removing solids when evaluated in jar tests, dissolved chitosan did show promise in an unexpected manner. When dissolved chitosan was added to the water discharged from a recirculating system before passing this flow through a fluidized-sand biofilter, the dissolved chitosan increased the capture of TSS within the expanded bed. Thus, adding dissolved chitosan to water flowing into a fluidized-sand biofilter turned the biofilter into a novel

type of upflow 'sludge blanket clarifier,' which appears to be both non-plugging (because it is a fluidized bed) and relatively simple to operate because it would never require backwashing. In addition, the dissolved chitosan did not affect the nitrification across the fluidized-sand biofilter. Therefore, there is potential for the over-topping effluent from a recirculating system to be treated for both dissolved wastes (e.g., TAN and soluble BOD) and TSS by adding low levels (~0.5 mg/L) of dissolved chitosan to the flow before it passes through a fluidized-sand biofilter.

Additional work is necessary to:

- Determine the effects of dissolved chitosan addition on long-term fluidized-sand biofilter operation, especially to validate the non-effect on nitrifying bacteria and to identify suitable bed management routines.
- Estimate the cost of chitosan addition in a full-scale commercial application.
- Ascertain how much dissolved chitosan passes through a fully developed fluidized-sand biofilter to determine if there would be potential for chitosan toxicity problems encountered after this effluent is discharged.
- Identify other polymers that are non-toxic to fish and humans and would coagulate TSS within fluidized-sand biofilters.
- Test alternate solvents for chitosan such as formic acid or malic acid, because chitosan dissolved with malic acid has been reported to be non-toxic to fathead minnows (Technical Data Sheet Sea Klear Chitosan Toxicity Data, 11/8/96, Vanson, Redmond, WA, USA).

ACKNOWLEDGMENTS

This work was supported by the United States Department of Agriculture, Agricultural Research Service under grant agreement numbers 59-1931-8-038 and 59-1931-3-012. We thank Graham Bullock, Alicia Noble, Michael Schwartz and Susan Glenn for their assistance. The experimental protocol and methods used in this study were in compliance with Animal Welfare Act (9CFR) requirements and are approved by the Freshwater Institute Institutional Animal Care and Use Committee.

REFERENCES

Anderson, D. P., Siwicki, A. K. Duration of Protection Against *Aeromonas salmonicida* Immunostimulated with Glucan or Chitosan by Injection or Immersion. *Progressive Fish-Culturist* **1994**. *56*, 258-261.

American Public Health Association (APHA) **1989**. Standard Methods for the Examination of Water and Wastewater, 17th Ed. American Public Health Association. Washington, DC, USA.

Bough, W. A. Chitosan—A Polymer from Seafood Waste, for Use in Treatment of Food Processing Wastes and Activated Sludge. *Process Biochemistry* **1976**. *11*, 13-16.

Bullock, G., Blazer, V., Tsukuda, S., Summerfelt, S. Toxicity of Acidified Chitosan for Cultured Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture* **2000**. *185*, 273-280.

Chapman, P. E., Popham, J. D., Griffin, J., Michaelson, J. Differentiation of Physical from Chemical Toxicity in Solid Waste Fish Bioassay. *Water, Air, and Soil Pollution* **1987**. *33*, 295-308.

Culp, R. L., Wesner, G. M., Culp, G. L. **1978**. Handbook of Advanced Wastewater Treatment, 2nd Edition. Van Nostrand Reinhold Company, New York, NY, USA.

Ebeling, J. M., Sibrell, P. L., Ogden, S. R., Summerfelt, S. T. Evaluation of Chemical Coagulation-Flocculation Aids for Sequestering of Phosphorus from Intensive Recirculating Aquaculture Effluent Discharge. *Aquacultural Engineering*. In review.

Elson, C. M. Agricultural and Medical Applications of N,O-carboxymethylchitosan, a Derivative of Shrimp Processing Waste. *Bulletin Aquaculture Association Canada* **1996**. *96*, 39-44.

Heinen, J. M., Hankins, J. A., Weber, A. L., Watten, B. J. A Semiclosed Recirculating-Water System for High-Density Culture of Rainbow Trout. *The Progressive Fish-Culturist*. **1996**a. *58*, 11-22.

Heinen, J. M., Hankins, J. A., Adler, P.R. Water Quality and Waste Production in a Recirculating Trout Culture System with Feeding of a Higher-Energy or a Lower-Energy Diet. *Aquaculture Research.* **1996**b. *27*, 699-710.

Kawamura, S. Effectiveness of Natural Polyelectrolytes in Water Treatment. *Journal of the American Water Works Association* **1991**. *83*, 88-91.

Kono, M., Matsui, T., Shimizu, C. Effect of Chitin, Chitosan, and Cellulose as Diet Supplements on the Growth of Cultured Fish. *Nippon Suisan Gakkaishi.* **1987**. *53*, 125-129.

Noble, A.C., Summerfelt, S.T. Diseases Encountered in Rainbow Trout Cultured in Recirculating Systems. *Annual Review of Fish Diseases* **1996**. *6*, 65-92.

Sandford, P. Chitosan: Commercial Uses and Potential Applications. In Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications. Skjak-Braek, G., Anthonsen, T., Sandford, P. (Eds.) **1989**. Pages 51-69. Elsevier Applied Science. New York, NY, USA.

Siwicki, A. K., Anderson, D. P., Rumsey, G. L. Dietary Intake of Immunostimulants by Rainbow Trout Affects Nonspecific Immunity and Protection Against Furunculosis. *Veterinary Immunology and Immunopathology* **1994**. *41*, 125-139.

Summerfelt, S. T., Hankins, J. A., Weber, A., Durant, M. D. Ozonation of a Recirculating Rainbow Trout Culture System: II. Effects on Microscreen Filtration and Water Quality. *Aquaculture* **1997**. *158*, 57-67.

Timmons, M. B., Ebeling, J. M., Wheaton, F. W., Summerfelt, S. T., Vinci, B. J. **2002**. Recirculating Aquaculture Systems, 2nd Edition. Cayuga Aquaculture Ventures, LLC. Ithaca, NY, USA.

Vaidya, M. V., Bulusu, K. R. Chitosan as a Coagulant and Coagulant Aid. *Journal of the Institution of Engineers* (India) **1984**. *64*, 43-48.

Weeks, N. C., Timmons, M. B., Chen, S. Feasibility of Using Foam Fractionation for the Removal of Dissolved and Suspended Solids from Fish Culture Water. *Aquacultural Engineering* **1992**. *11*, 251-265.

Organoleptic, Chemical and Microbiological Changes in European Eel (*Anguilla anguilla* L.) During Storage: Skinned vs. Unskinned Fish held at 2°C and 5°C

E. McLean[1]*, B. Rønsholdt[2], R. Roesen Nørgaard[2], C. Kjølby Jensen[2], L. Jacobsen[3]

[1]*Virginia Polytechnic Institute and State University Aquaculture Center
Department of Fisheries and Wildlife Sciences,
156 Cheatham Hall, Blacksburg, VA 24061-0321 USA
E-mail: emclean@vt.edu

[2] Aalborg University
Sohngaardsholmsvej 57
DK-9000 Aalborg, Denmark

[3] Heilsufrøðiliga Starvsstovan
Debesartrøð,
FO-100 Tørshavn, Faeroe Islands

* Corresponding Author

ABSTRACT

Studies were undertaken to examine quality deterioration in stored skinned and unskinned fresh eels derived from a commercial recirculation facility. Fish samples were held at 2 and 5°C for 18 days and examined sensorially, chemically and microbiologically. Oil content of experimental animals averaged 39.1%, that for dry matter was 47.8% and 12.1% and 0.69% for protein and ash content respectively. Trimethylamine-oxide (TMA-O) and trimethylamine (TMA) levels remained below detection limits (< 0.7 mg N 100 g^{-1}) throughout the study. Shelf life was extended for both skinned and unskinned fish at the lowest temperature. By day 14 of the trial, differences ($P < 0.05$) were detected by sensorial evaluation for both temperatures evaluated and between skinned and unskinned animals. Increases ($P < 0.05$) were detected for TVB-N throughout storage with fish held at 5°C expressing higher levels ($P < 0.05$). Sample lipid oxidation increased throughout the study period. TBA was determined as a suitable variable for predicting overall impression of product quality. K1 value illustrated significant ($P < 0.0001$; R^2 0.72) polynomial correlation with overall impression.

INTRODUCTION

European eel (*Anguilla anguilla*) production has expanded significantly over the last 15 years, with the sector experiencing a more than doubling in production from circa 4,500 metric tons in 1987 (Heinsbroek 1991) to 10,215 metric tons in 2001 (FEAP 2002). The major part of industry expansion has been achieved due to the development of intensive recirculation systems, together with an enhanced understanding of the nutritional and biological requirements of the species. Increased production however, has been accompanied by a decline in value, which has slumped by 42% $kilo^{-1}$ since 1995 (FEAP 2002). For the industry to remain viable, farmers must look towards further improvements in production efficiency and market diversification. At present, European eels are primarily used for the manufacture of value added semi-preserved products which include smoked and jellied eels. More recently, European eels have been used for the production of kabayaki destined for Japan and European specialty markets (Bovbjerg 1999, Byrne 1999). However, surveys have indicated that Dutch, French, and German markets in particular, have significant demands for fresh eels, with farmed animals generally being favored by the consumer due to their thinner skin and higher fat levels (Fransu 1989, Globefish 1998).

The development of an expanded fresh eel market will demand an increased awareness of the shelf life of such products. Only one previous study has considered the spoilage characteristics of fresh eel (Rehbein and Hinz 1983) and these authors indicated a shelf life of approximately 16 days for ice-stored wild fish, derived from the Baltic Sea. After this period however, organoleptic spoilage of the raw material became pronounced. In contrast to cultivated eels, wild fish have a lower fat content (Lie et al. 1990). Hence, lipid oxidation processes may be more prominent in farmed eels than their wild counterparts (Hutlin 1992). Such a difference may alter the shelf life characteristics of aquacultured animals in terms of chemical, bacterial and organoleptic changes to the flesh. In order to examine this possibility, the present study explored quality changes to aquacultured eels derived from a commercial intensive recirculating system. Both unskinned and skinned animals were investigated since, in the latter, oxygen and spoilage organisms may penetrate into the flesh more readily, accelerating deterioration of the raw material. The shelf life of fresh eel was evaluated at temperatures mimicing those encountered in the retail chain.

MATERIALS AND METHODS

Experimental

Animals

Eels reared in a recirculating system at 25°C were purchased from a commercial supplier (Milbak Eel Farm, Sulsted, Denmark). Fish, which were presumably of mixed sex, ranged between 140 and 160 g in weight at the time of sampling. All animals were fed Ecoline 19 pellets (Biomar, Brande, Denmark) throughout the production period but were purged for 7 days prior to slaughter. Eels were killed, gutted and either left intact or skinned at the farm, before transportation to the laboratory. Approximately 1 hour elapsed between slaughter and storage.

Storage and sensorial evaluations

Skinned and unskinned fish were placed in individual plastic bags and stored at 2°C and 5°C (+0.1°) for up to 18 days. At 0, 5, 10, 14 and 18 days, two eels from each treatment were evaluated microbiologically for H_2S-producing spoilage, and total bacteria. Total volatile base nitrogen (TVB-N), lipid oxidation (thiobarbituric acid {TBA}) and changes in flesh pH were also monitored. Sensory panels evaluated samples at 0, 5, 10, 14 and 18 days for both temperatures employed.

Analytical techniques

Sensory scheme and protocol development

Prior to sensorial evaluation, a sensory scheme was developed for skinned and unskinned raw eels stored at 2°C for 23 days. At various time intervals, raw and cooked eels were examined for organoleptic characteristics. Odor, flavor and textural parameters were incorporated into a descriptive profiling sensory scheme (Table 1), with a subjective scale describing overall impression. The sensory panel was provided with an instruction sheet that presented definitions for each parameter considered (Table 2). In order to minimize bias due to sample preparation several methods of cooking were examined prior to organoleptic assessment. These included preparing samples by heating at 90°C in PE/PA 20/70 bags, sealed under vacuum, for 5, 10, 15, 20 and 25 minutes or by baking at 175°C in covered aluminum trays for 10 and 15 minutes. Drip-loss from each sample was recorded during preparation. Samples prepared at 90°C for 20 minutes were chosen for all sensory analyses since

this method did not produce detectable off-odors or flavors and cooking for 20 minutes resulted in all samples being fully prepared, with a drip loss of 17-20%. In contrast, cooking for 25 minutes yielded a 25% drip-loss, whereas baking in covered aluminum trays for 10 or 15 minutes led to sample drying, discoloration and production of an 'oily' odor.

Sensory analyses

A trained panel of six performed sensory analyses. Sample designation codes were randomized. A small cutlet, approximately 25 g wet weight, was served immediately following preparation. Smell, taste, and texture were evaluated by means of the developed sensory scheme (Table 1). An overall impression of "less acceptable" was set as a rejection point while shelf life was defined as the point where at least half of the panelists rejected the sample. Loss of prime quality was defined as the point where at least half of the panelists judged the fish "less good" (Table 1).

Microbiological analyses

Iron Agar (IA) was used for total aerobic and H_2S-producing spoilage bacteria counts (Veterinaerdirektoratet 1989). Spread and pour plates were used. All tissue samples for analyses were taken between the dorsal fin and lateral line posterior to the vent. Lactic acid-forming bacteria were measured as pour plate count in nitrite actidione polymyxin agar (NAP) (Davidson and Cronin 1973), using APT agar supplemented with 1ml $100ml^{-1}$ of NAP solution (Merck KGaA, Darmstadt, Germany). For surface counts a 5 cm^2 area (approximately 1 mm thick) of skin was used. For deep flesh counts, the surface was sterilized by heating, and a 5 g sample (~ 2 cm^3) removed, homogenized (Colworth stomacher, Seward, London, UK) in 0.9% NaCl with 0.1% peptone for 2 minutes and a dilution series constructed followed by inoculation. Plates were aerobically incubated for 72 hours at 21°C for total and H_2S-producing bacteria and for 120 hours at 21°C for lactic acid-forming bacteria (Veterinaerdirektoratet 1989) before counting.

Chemical analyses

All chemical analyses were performed in duplicate for each sample. Fish were filleted and homogenized in a blender. Dry matter and ash content of the fillet was determined after 24 hours at 105°C and 550°C respectively. Oil content of was determined using chloroform:methanol extraction (Bligh and Dyer 1959), and protein content (Kjeldahl-N)

Table 1. Sensory panel scheme developed and employed for the evaluation of cooked eels. See Table 2 for detailed explanation of each of the definitions employed.

Sensory evaluation of cooked eel

Oil	Smell	Fresh	Neutral	Slight train oil-like	Train oil to rancid	Rancid	Very rancid
Off-smell	Hay-/muddy	None	Slight	Moderate	High		
	Sour	None	Slight	Moderate	High		
	NH_4-like	None	Slight	Moderate	High		
	Yeast-like	None	Slight	Moderate	High		
	Spoiled	None	Slight	Moderate	High		
Other							

Name:_____ Date:_____ Code:_____

Oil	Taste	Fresh	Neutral	Slight train oil-like	Train oil to rancid	Rancid	Very rancid
Off-taste	Muddy	None	Slight	Moderate	High		
	Bitter	None	Slight	Moderate	High		
	NH_4-like	None	Slight	Moderate	High		
	Sour	None	Slight	Moderate	High		
	Spoiled	None	Slight	Moderate	High		
Other							

Table 1. cont'd.

Texture		**Fresh**	**Neutral**	**Slight train oil-like**	**Train oil to rancid**
	Elasticity	None	Slight	Moderate	High
	Firmness	None	Slight	Moderate	High
	Toughness	None	Slight	Moderate	High
	Juiciness	None	Slight	Moderate	High
	Grainy/grittiness	None	Slight	Moderate	High
	Stickiness	None	Slight	Moderate	High
Other					

Overall impression	Very good	Good	Less good	Acceptable	Less acceptable	Unacceptable	Poor

Comments: _____

_____

_____

_____

Definitions and Instructions

Smell: Smell the sample in the plastic bag immediately after opening.

- An oil smell is characterized by: fresh oil smell is pleasant, train oil-like smell is unpleasant, rancid oil smell is acrid, thick and very unpleasant.
- Hay like smell is characterized by: cut, dried grass odor
- Yeast-like smell is characterized by sickly sweet and moldy odor
- Ammonia-like smell characterized by prickly odor
- Spoiled smell, characterized by a stuffy to rotten and unpleasant odor

Taste: Before tasting, the skin and/or sub-dermal layer is removed and a sample of white dorsal flesh used.

- Oil taste characterized by same parameters as for smell
- Muddy off-taste, recognized 5-10 seconds after the sample is placed in the mouth
- Ammonia-like taste detected as a very prickly feeling
- Spoiled taste is characterized by a tainted to rotten and unpleasant flavor

Texture: The texture is defined for the individual parameters. A sample of white dorsal flesh is used.

- Elasticity is described immediately after the sample is placed into the mouth. The flesh is considered elastic when it "bounds" back after one or two chews.
- Firmness is when the flesh feels cohesive after several chews.
- Toughness is when the flesh is continuously tough after a minimum of 5 chews (rubber like).
- Juiciness is when the flesh maintains moisture, thus not drying after 5 chews.
- Graininess/grittiness is when the flesh feels mushy and incohesive
- Stickiness is when the flesh sticks to the teeth and a resistance is experienced when chewing after 4-5 chews.

Overall impression: This is judged as the impression in comparison to other eels served during training at this and earlier sessions. Impression does not depend upon personal affinity for eel. When all parameters are good, the fish is judged as being very good. Slight off tastes reduce overall impression to less good, etc.

Comment: Describe why the fish has received the respective score in overall impression; e.g., if a muddy taste results in a lower score.

Level of score:

- None: is where the stated characteristics are undetectable.
- Slight: is where the respective characteristics can be traced but not in a pronounced manner
- Moderate: is where the presence of a characteristic is unequivocal
- High is where the characteristic is strongly present

Table 2. Instructional sheet employed during the sensorial evaluation of cooked eels, providing definitions and instructions to sensory panelists.

according to AOAC (1984). TBA was determined as described by Vyncke (1975) with the following modification: a 15 g sample was mixed in 40 ml trichloracetic acid solution and incubated for 24 hours at 21°C. Absorbency was read at 530 nm. Drip-loss was measured as the difference between initial weight and actual weight of the eel. TVB-N, TMA-O and TMA were determined using Conway micro diffusion chambers (Conway and Byrne 1933) with 0.025 N HCL in the inner ring and saturated K_2SO_4 in the outer ring. Volatile bases were extracted from the sample in a 1:4 mixture by weight of homogenized fish flesh and distilled water, pH adjusted to 5.2, and heated to 70°C for 2 minutes. pH was measured in the mixture at 25°C prior to adjustment with HCl. Nucleotide breakdown was measured as a k_1 value (Gill 1992) using Fresh tester FTP II sticks (Transia, EAC Corporation, Japan). All chemicals used were of analytical grade, obtained through Merck, except substrates for IA plates (Difco, Detroit, MI, USA).

Data analyses

Chemical and microbial data were analyzed statistically using two way ANOVA, and pairwise Student Newman-Keul comparison tests were used to test for differences between groups. Correlations between overall impression and TBA/k_1 values were examined using least square regression. Data from the sensory panel were analyzed by means of multivariate calibration using UNSCRAMBLER® (Camo, Trondheim, Norway). Each parameter on the sensory sheets was given a score, corresponding to sensory score specified the level detected, *e.g.* 0 = none, 1 = slight, 2 = moderate, 3 = high (Figure 1). Correlation coefficients between overall impression (Y-matrix) and various groups (X-matrix) were assessed using a Partial Least Square model (PLS1). Resulting weighting coefficients (B_W-matrix) display the significance of each examined parameter in describing overall impression. Thus, higher absolute value indicates the most important parameters. Positive weighting coefficients reveal a positive correlation between the parameter and overall impression, whereas negative coefficients infer the opposite. Comparison of treatments was undertaken using PLS2 models, correlating sensory characteristics (X-matrix) to overall impression (Y-matrix). Dummy variables were included in the Y-matrix (as an identity matrix), with each dummy vector given a value 1 for one respective code at a given time, while remaining groups were allocated value 0 in the same vector. Resulting loading plots for the dummy variables revealed

differences between groups related to sensory characteristics. All raw data were weighted by $STDEV^{-1}$ prior to PLS modeling.

RESULTS

The average oil content of eel was 39.1% (n=8; range: 31-47%), whereas dry matter was 47.8% (n=8; range: 43.7-66.6%). Protein and ash content were 12.1% (n=8; range 11.6-13.1%), and 0.69% (n=8; range 0.64-0.73%) respectively. TMA-O and TMA in eels stored for 0 or 13 days (n= 4) were below limits of detection (< 0.7 mg N $100g^{-1}$).

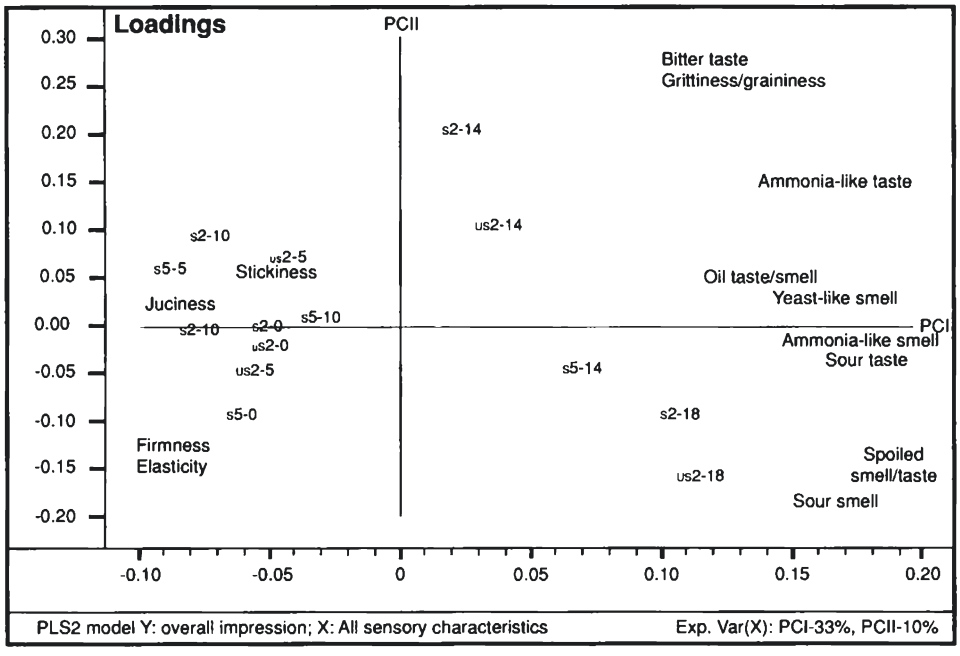


Figure 1. Trends in sensory characteristics among groups and days shown by PLS2 plots. us2: unskinned fish stored at 2 ℃; us5: unskinned fish stored at 5 ℃; s2: skinned fish stored at 2 ℃; s5: skinned fish stored at 5 ℃. The hyphenated number following each point represents the number of days the sample was stored.

Sensory changes and shelf life

The effects of temperature and skinning upon shelf life, maintenance of quality, and overall impression are summarized in Table 3. Prime quality was maintained for a period of 5 days irrespective of storage temperature or the presence of skin. Shelf life was extended for both skinned and unskinned eels at the lower temperature employed (Table 3). No differences in overall impression were recorded between treatments for the first 10 days of storage. By day 14 however, diffcrences ($P < 0.05$) in overall impression became apparent for both temperatures evaluated and for skinned and unskinned fish. By day 18 of the trial, eel stored at 5°C were considered unacceptable. Fish stored at 2°C were considered acceptable but expressed a significant decline in overall impression when compared against all other sampling points (Table 3). PLS2 plots (Figure 1) revealed trends in changes to sensory characteristics for the eels during storage, with PCI explaining 33% and PCII 10% of the variance. Table 4 notes weightings of the sensory characteristics examined. The first signs of spoilage were changes in the smell of oil together with a train oil-like taste. Texture characteristics changed only slightly during the first 10 days of storage with the sensory panel being unable to determine the presence of off-flavors/odors. Textural changes and off-odors/flavors became prominent from day 10 onwards, mainly being expressed in unskinned eels as a loss of firmness, development of a sticky flesh structure and increased graininess/grittiness. The sensory characteristics changed more rapidly in fish stored at 5°C than those stored at 2°C, with differences being apparent at day 14. This resulted in eels stored at 2°C having a longer shelf life than those stored at 5°C. The impact of skinning also became apparent following 10-14 days of storage, with skinned eels expressing unfavorable organoleptic characteristics when compared against unskinned samples. A muddy smell/taste was detected in some eels and panelists commented that this was unappealing. However, since this flavor did not change over time, no correlation was found with spoilage or degradation.

Chemical analyses

Significant ($P<0.05$) increases in TVB-N (Figure 2) were observed throughout storage, with eels held at 5°C expressing higher levels ($P<0.05$) than fish maintained at 2°C from day 10 onwards. Unskinned animals exhibited elevated levels of TVB-N ($P<0.05$) when compared to skinned fish (Figure 2). As for TVB-N, the degree of sample lipid

	2°C		5°C	
	Skinned	**Unskinned**	**Skinned**	**Unskinned**
Prime quality (days)	5	5	5	5
Range	(0 - 5)	(0 - 5)	(0 - 5)	(0 - 5)
Shelf life (days)	18	18	14	14
Range	(14 - 18)	(14 - 18)	(10 - 14)	(10 - 14)
Overall impression*:				
Day 0	1.20[a]	1.20[a]	1.80[a]	1.80[a]
Day 5	1.60[a]	2.00[a]	1.40[a]	2.20[a]
Day 10	1.40[a]	1.80[a]	2.40[a]	2.20[a]
Day 14	3.40[b]	3.40[b]	4.00[b]	5.20[b]
Day 18	5.60[c]	5.60[c]		

* *SEM = 0.52*

Table 3. Maintenance of prime quality, shelf life and changes in overall impression (0 = very good; 6 = poor) of skinned and unskinned European eels (n = 5) stored at 2 °C and 5 °C. Similar superscripts in the same column indicate no significant difference (P > 0.05) between. No differences were found between groups in terms of quality, shelf life and changes in overall impression.

oxidation (TBA) also increased throughout the study period (Figure 3), with differences ($P < 0.05$) in TBA presence being apparent by day 5 between skinned and unskinned eel maintained at the same temperature. TBA was the most suitable variable for predicting overall impression (PLS1 modeling between overall impression and chemical/microbial analysis) with strong polynomial correlation ($P<0.0001$; $R^2=0.75$) being determined.

The response of flesh pH to different storage temperatures and the presence of skin is summarized in Figure 4. Skinned eels exhibited a biphasic increase in flesh pH, with rapid initial increases ($P<0.05$) in pH, when compared against unskinned eels, followed by a plateau at approximately pH 6.75 to trial termination. In contrast, unskinned eels expressed a continuous increase ($P<0.05$) in pH throughout the experiment, with final values being recorded as approximately pH 7.5.

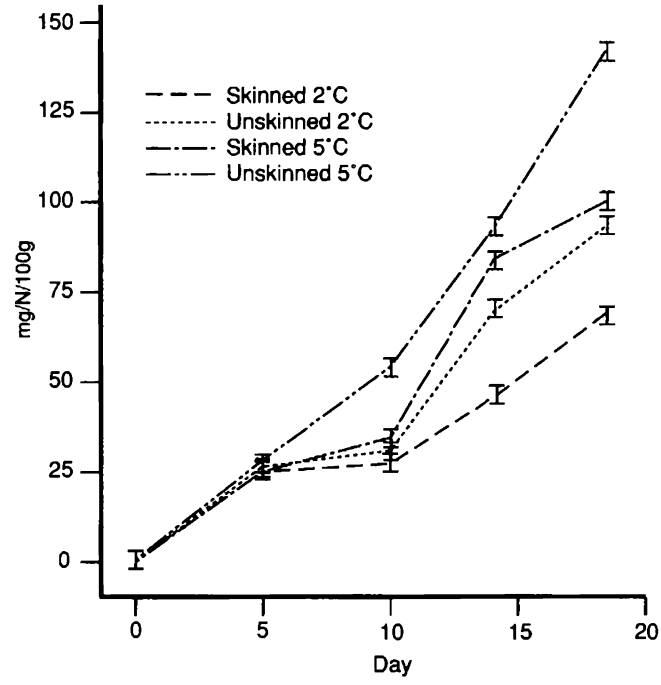


Figure 2. Development of Total Volatile Bases-Nitrogen (TVB-N) in skinned and unskinned European eel stored at 2 ℃ or 5 ℃. Vertical bars represent ± SEM (n = 2).

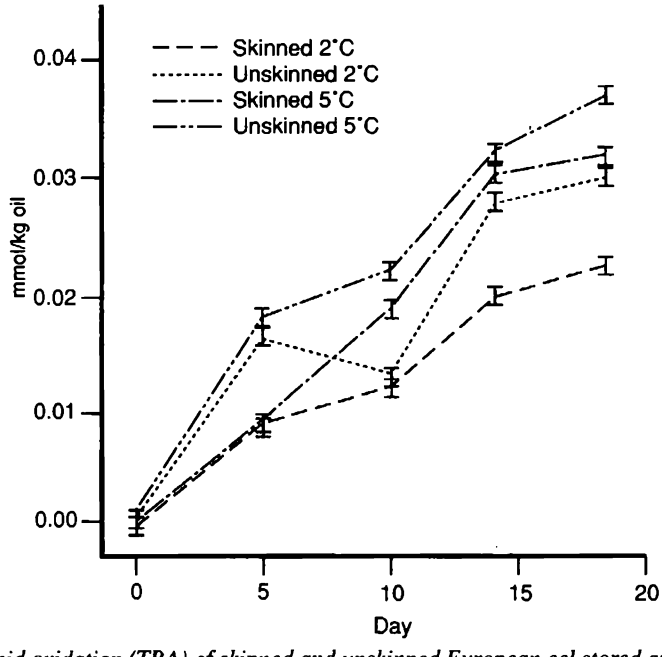


Figure 3. Lipid oxidation (TBA) of skinned and unskinned European eel stored at 2 ℃ or 5 ℃. Vertical bars represent ± SEM (n = 2).

Measurement of nucleotide breakdown in skinned eels stored at 2°C (Figure 5) revealed a significant increase ($P<0.05$) over time with $k_1 = 51.25$ at rejection (day 18). The k_1-value demonstrated a significant ($P<0.0001$; $R^2 = 0.72$) polynomial correlation with overall impression.

Microbiological analyses

Spread plates (n=160) had significantly ($P<0.05$) higher bacterial counts than pour plates: differences were usually 40-50% higher for the spread plates but extremes of up to 90% were seen. Hence, only results obtained with spread plates were considered in later analyses. Initially 10^4-10^5 bacteria cm^{-2} were present on the surface of the eels, with no H_2S-producing spoilage bacteria being detected (Table 5). Bacteria developed exponentially ($P<0.05$) during the first 10 days of storage, followed by a stationary phase, which lasted for the remainder of the trial. Bacterial counts in eels stored at 5°C increased more rapidly than those stored at 2°C ($P<0.05$), and unskinned fish had a more rapid increase in bacterial numbers than skinned eels (Table 5).

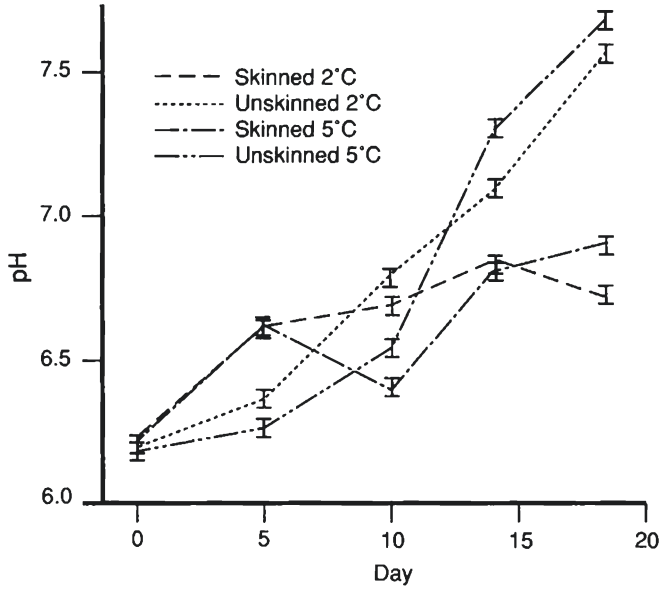


Figure 4. Changes in flesh pH of skinned and unskinned European eel stored at 2 °C or 5 °C. Vertical bars represent ± SEM (n = 2).

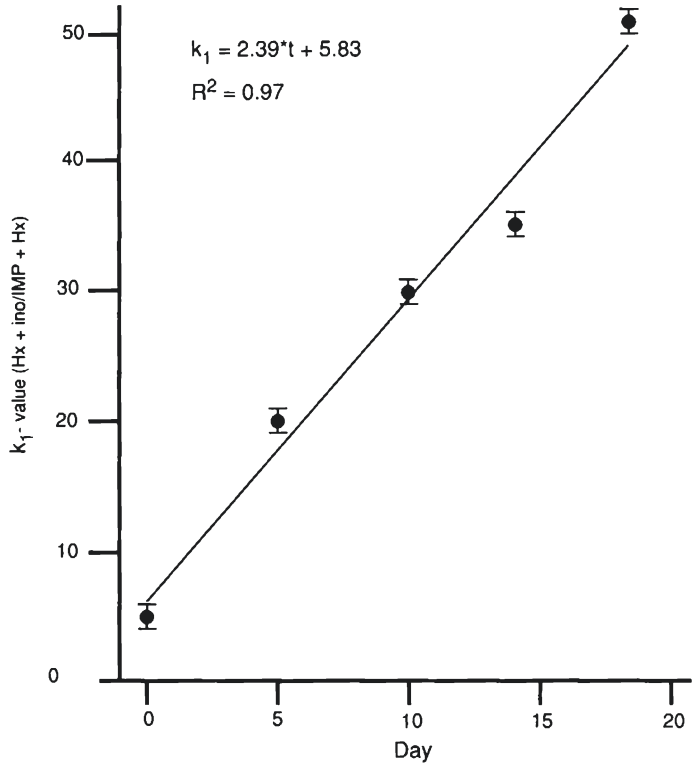


Figure 5. Nucleotide breakdown (k_1) in skinned eels stored at 2 °C. IMP was the level of inosine monophosphate, Hx the level of hypoxanthine, and Ino the level of inosine. Vertical bars represent ± SEM (n = 2)

DISCUSSION

The eels used in the present study were raised under intensive recirculating conditions and expressed fat content 2.5 - 3.5 times higher than those reported for wild fish (Rehbein and Hinz 1983, Lie et al. 1990) and 1.0 - 1.8 times those reported previously for farmed eels (Lie et al. 1990, Gallego et al. 1993). These differences might be due to feeding: the eels used in the present investigations had been fed a high-energy diet (23% lipid) and body fat content is influenced by dietary fat level (Coello et al. 1999, Rasmussen et al. 2000). The shelf life determined for fresh farmed chilled eels was in general agreement with that reported by Rehbein and Hinz (1983) for raw, wild eels. The latter authors indicated a shelf life of 16-20 days for fish stored at 0°C. Thus, it appears that farmed eels, even with a 3-fold higher fat content, maintain quality for as long as wild animals when taking storage temperature differences into account. In the present study, temperature had a greater

Correlation coefficient R^2		0.7852
Sensory variables		**Parameter weight**
Smell	Oil	0.2265
	Muddy	0.0704
	Sour	0.0872
	Ammonia-like	0.0367
	Fermented	-0.0947
	Spoiled	0.6243
Taste	Oil	0.3381
	Muddy	-0.0425
	Bitter	0.0477
	Ammonia-like	0.1697
	Sour	0.0005
	Spoiled	0.5249
Texture	Elasticity	-0.0750
	Firmness	-0.3424
	Toughness	0.0943
	Juiciness	-0.0346
	Grainy/gritty	0.4957
	Sticky	0.2423

Table 4. Correlation between overall impression and sensory variables (mean values, n = 20) for chill stored eels (PLS1 model). Parameter weight indicates importance of sensory attributes.

Total surface bacterial counts*					Total deep flesh bacterial counts**			
Day	2°C S	2°C US	5°C S	5°C US	2°C S	2°C US	5°C S	5°C US
0	5.35_{A}^{a}	4.42_{A}^{b}	5.35_{A}^{a}	4.42_{A}^{b}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}
5	6.46_{B}^{a}	6.93_{B}^{b}	7.45_{B}^{c}	7.85_{B}^{d}	3.45_{B}^{a}	4.37_{B}^{b}	4.40_{B}^{b}	5.3_{B}^{c}
10	8.92_{C}^{a}	$9.28_{C}^{b,c}$	$9.10_{C}^{a,b}$	9.36_{C}^{c}	6.48_{C}^{a}	7.01_{C}^{b}	$7.11_{C}^{b,c}$	7.43_{C}^{c}
14	$9.54_{D}^{a,c}$	9.68_{D}^{a}	9.33_{C}^{b}	$9.44_{C}^{a,b,c}$	7.32_{D}^{a}	8.07_{D}^{b}	6.93_{C}^{c}	6.93_{D}^{c}
18	9.90_{E}^{a}	$9.49_{C,D}^{b}$	9.23_{CD}^{c}	9.89_{D}^{a}	6.58_{C}^{a}	7.10_{C}^{b}	7.09_{C}^{b}	7.26_{CD}^{b}
H_2S-producing bacterial counts (surface)*					H_2S-producing bacterial counts (deep flesh)***			
Day	2°C S	2°C US	5°C S	5°C US	2°C S	2°C US	5°C S	5°C US
0	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}	ND_{A}^{a}
5	3.83_{B}^{a}	4.77_{B}^{b}	6.06_{B}^{c}	7.14_{B}^{d}	1.00_{B}^{a}	2.74_{B}^{b}	3.37_{B}^{c}	4.32_{B}^{d}
10	7.32_{C}^{a}	8.05_{C}^{b}	7.40_{C}^{a}	8.18_{C}^{b}	4.85_{C}^{a}	5.64_{C}^{b}	5.73_{C}^{b}	6.24_{C}^{c}
14	7.84_{D}^{a}	$8.28_{C,D}^{b}$	7.84_{D}^{a}	8.70_{D}^{c}	5.34_{D}^{b}	6.20_{C}^{c}	5.92_{C}^{c}	6.32_{C}^{b}
18	7.98_{D}^{a}	8.40_{D}^{b}	7.19_{E}^{c}	8.46_{E}^{b}	5.81_{C}^{b}	5.33_{D}^{b}	6.51_{D}^{b}	6.06_{C}^{c}

* SEM = 0.05

** SEM = 0.08

*** SEM = 0.09

Table 5. Development of total surface (log CFU/cm²), deep flesh (log CFU/g), and H_2S-producing bacteria in skinned (S) and unskinned (US) eels held at 2 °C and 5 °C (n = 2). ND = not detectable. Values in the same column exhibiting different subscripts were significantly different ($P < 0.05$). Similar superscripts in the same row, for each of the bacterial counts, indicate no difference ($P > 0.05$) between counts.

influence over organoleptically detectable spoilage of eel than did skinning.

Organoleptic, microbiological and biochemical indices were all affected by temperature: more rapid spoilage was observed at high temperature, and this increased colonization by H_2S-producing bacteria (Table 5), elevated TBA/TVB-N (Figs. 2 and 3) and enhanced rejection by the sensory panel (Table 3). All the preceding factors are well-documented occurrences during seafood spoilage (see Sikorski et al. 1990, Huss 1995, Bremner 2002). Skinning was correlated with lower bacterial loading, decreased lipid oxidation and retarded TVB-N appearance profiles. Unskinned fish initially expressed lower flesh pH values than skinned eel, which likely resulted due to anaerobic

conditions restraining the breakdown of muscle lactic acid (Huss 1995). In the present study, a change in flesh pH (from 6.2-7.6) was associated with alterations in flesh firmness and the onset of a gritty/grainy texture. A lowering of flesh pH is generally associated with a weakening of structural elements by different endogenous muscle peptidases (calpain/ cathepsin) that degrade myofibrillär components and its breakdown products (see Dransfield 1994, Davis et al. 1994).

Some eel samples expressed a muddy flavor, which was classed by the sensory panel as an unappetizing feature, decreasing overall impression. In an attempt to clear this off-flavor, eels were starved and purged in fresh water for a week. In catfish and other species, this period of time is generally considered as adequate for the purpose (Heikes 1993, Persson 1995). However, with eel, the variable muddy flavor was maintained even after purging, which may be one reason underlying the differences in acceptability noted for some of the fresh eels on day 0 of the study. Thus a longer period of starvation and purging may be necessary to gain excellent initial quality ratings by sensory panels, although further investigations in this field should be undertaken to establish optimal conditions for purging.

The existence of discrete connections between sensory, microbial and or biochemical indices are of high importance, since these may assist in constructing quality grading systems for industrial application. From the results presented, TBA and or k_1 values may be useful as estimators of chilled stored eel quality. The k_1 value increased linearly over time, with a level of approximately 50 at the point of rejection. These data correlate well with that presented for yellowtail (Sakaguchi and Koike 1992), within the first 10 days of chill storage but differ from the findings of Rodriguez et al (1999) with refrigerated (4-5°C) rainbow trout following 12 days storage. The latter authors indicated a k_1 value of 70 on the day of marginal acceptability. From the results presented, farmed unskinned eels held at 2°C, provided the most favorable storage conditions, with a shelf life of 14-18 days. However, prime quality was lost after 5 days.

ACKNOWLEDGEMENT

The authors wish to thank members of the sensory panel for participating in the study.

REFERENCES

Association of Analytical Communities (AOAC) **1984**. Official Methods of Analysis, 14th Edition. AOAC, Arlington, VA, USA.

Bligh, E. G., Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology* **1959**. *37*, 911-917.

Bremner, H.A. (Ed.) **2002**. Safety and Quality Issues in Fish Processing. Woodhead Publishing Ltd., Abington Hall, Abington, Cambridge, UK.

Bovbjerg, P. Eel Culture Trends in Europe. *Fisheries Bulletin of the Marine Institute* (Dublin) **1999**. *17*, 35-37.

Byrne, B. Eel Products and Markets: An Overview of the Market for Eel in Germany. *Fisheries Bulletin of the Marine Institute* (Dublin) **1999**. *17*, 39-41.

Coello, M. T., Sanchez, M. -J., Vicario, I. M., Heredia, F. J., Martin, M. Assessing Acceptability of Eel (*Anguilla anguilla*) Fed Three Different Diets. *Journal of the Science of Food and Agriculture* **1999**. *79*, 2087-2093.

Conway, E.J., Byrne, A. An Absorption Apparatus for the Micro-Determination of Certain Volatile Substances - I: The Micro-Determination of Ammonia. *Biochemical Journal* **1933**. *27*, 419-429.

Davidson, C. M., Cronin, F. Medium for the Selective Enumeration of Lactic Acid Bacteria from Food. *Applied Microbiology*. **1973**. *26*, 439-440.

Davis, J. R., Ledward, D. A., Bardsley-Ronald, G., Poulter, G. Species Dependence of Fish Myosin Stability to Heat and Frozen Storage. *International Journal of Food Science and Technology* **1994**. *29*, 287-301.

Dransfield, E. Modeling Post-Mortem Tenderisation: vs. Inactivation of Calpains. *Meat Science* **1994**. *37*, 391-409.

Federation of European Aquaculture Producers (FEAP). European Eel Production. http://www.feap.org/eels.html. **2002.**

Fransu, L. World Eel Market Review. *INFOFISH International* **1989**. *6*, 18-22.

Gallego, G. G., Hidalgo, M. C., Suarez, M. D., Sanz, A., Higuera, M. Feeding of the European Eel *Anguilla anguilla*. II Influence of Dietary Lipid Level. *Comparative Biochemistry and Physiology* **1993**. *105A*, 171-175.

Gill, T. A. Biochemical and Chemical Studies on Seafood Quality. In Quality Assurance in the Fish Industry. Huss, H. H. (Ed.). **1992**. Elsevier Science Publishers B.V., Amsterdam, Holland.

Globefish. The Markets for Freshwater Fish in Europe. Globefish Research Programme **1998**. *49*.

Heikes, D. In-Pond Purging for Catfish with Off-Flavor. *Aquaculture Magazine* **1993**, *July/August*, 28-32.

Heinsbroek, L. T. N. A Review of Eel Culture in Japan and Europe. *Aquaculture and Fisheries Management* **1991**. *22*, 57-72.

Hultin, H. O. Biochemical Deterioration of Fish Muscle. In Quality Assurance in the Fish Industry. Huss, H. H. (Ed.). **1992**. Elsevier Science Publishers B.V., Amsterdam, Holland.

Huss, H. H. **1995**. Quality and Quality Changes in Fresh Fish. FAO, Rome, Italy.

Lie, Ø., Hemre, G. I., Lambertsen, G. A Comparison of the Composition of Cultured and Wild Caught European Eel (*Anguilla anguilla*), Particularly Regarding Lipids. *Fiskeridirektoratet Skr., Series Ernæring* **1990**. *III*, 3-11.

Rehbein, V. H., Hinz, A. Vergleich Zwischen Physikalischen und Chemischen Metoden zur Bestimmung des Verderbs Unbehandelter oder Entschleimter aal bei Eislagerung. *Archiv fûr Lebensmittelhygiene* **1983**. *34*, 53-80.

Persson, P. E. 19th Century and Early 20th Century Studies on Aquatic Off-Flavours - A Historical Review. *Water Science and Technology* **1995**. *31*, 9-13.

Rasmussen, R. S., Ostenfeld, T. H., McLean, E. Growth and Feed Utilisation of Rainbow Trout Subjected to Changes in Feed Lipid Concentrations. *Aquaculture International* **2000**. *8*, 531-542.

Rodriguez, C. J., Besterio, I., Pascual, C. Biochemical Changes in Freshwater Rainbow Trout (*Oncorhynchus mykiss*) During Chilled Storage. *Journal of the Science of Food and Agriculture* **1999**. *79*, 1473-1480.

Sakaguchi, M., Koike, A. Freshness Assessment of Fish Using the Tory Meter and K-Value. In Quality Assurance in the Fish Industry. Huss, H. H. (Ed.). **1992**. Elsevier Science Publishers, Amsterdam, Holland.

Sikorski, E. Z., Kolawski, A., Pan, B. S. Post Harvest Biochemical and Microbial Changes. In Seafood: Resources, Nutritional Composition and Preservation Sikorski, E. Z. (Ed.). **1990**. CRC Press, Inc. Boca Raton, Florida, USA.

Veterinærdirektoratet, Cirkulære om mikrobiologiske undersøgelser af levnedsmidler. **1989**. Veterinærdirektoratet, Copenhagen, Denmark.

Vyncke, W. Evaluation of the Direct Thiobarbituric Acid Extraction Method for Determining Oxidative Rancididy in Mackerel (*Scomber scombrus* L.). *Fette, Seifen, Anstrichmittel* **1995**. *77*, 239-240.

Fish Nutrition (Third Edition)

J.E. Halver, R.W. Hardy (Eds.).
Academic Press, San Diego, CA, USA. 2002. XIV + 824 pp.
ISBN 0-12-319652-3. US$199.95

Reviewed by: Stephen Goddard
Department of Marine Science and Fisheries
College of Agricultural and Marine Sciences
Sultan Qaboos University
Sultanate of Oman
E-Mail: sgoddard@squ.edu.om

The first edition of Fish Nutrition, edited by J. E. Halver, was published in 1972. It brought together reviews from specialists across a broad range of basic and applied topics relating to the feeding and nutrition of fish. Publication coincided with a resurgence of interest in aquaculture and the book became established as a key reference source in a rapidly expanding field. An enlarged second edition was published in 1989. In producing a third edition, the editors have sought to incorporate selections of the most important new information produced since 1989. A single additional chapter has been contributed by the editors and there are new emphases and treatments in several chapters, which reflect reworking and changes in authorship.

The book opens with a chapter contributed by Bureau, Kaushik and Cho, which deals with the topic of bioenergetics. This chapter has been rewritten and expanded to reflect the growth of research activity in this field. Emphasis is on fish bioenergetics in an aquaculture setting and topics lead the reader in a logical sequence from historical review through to current perspectives and limitations. The contents of Chapters 2-6 follow those of earlier volumes giving detailed descriptions of the nutrient requirements of fish. Vitamin requirements are reviewed by Halver in Chapter 2, amino acid and protein requirements by Wilson in Chapter 3, lipid requirements by Sargent, Tocher and Bell in Chapter 4 and mineral requirements by Lall in Chapter 5. These are all solid contributions from leading specialists in their fields and these opening chapters form the core of the book. Whilst

much of this basic information is now widely available in books and reviews elsewhere, the authors have performed a useful task in their review of recent literature. The chapter dealing with lipid requirements deserves special mention. Written in an engaging style, the authors have reviewed the topic in depth and expanded the sections dealing with the lipid requirements of marine fish and larvae.

In Chapter 6, Dabrowski and Guderley review intermediary metabolism. This is a contribution by new authors who examine in detail the metabolism of carbohydrates and proteins. Nutritional physiology is reviewed by Rust in Chapter 7. The diversity of form and function of the fish digestive tract is described and an expanded section covering larval fish is included. Liberal use is made of b/w photographs whilst readers are directed to a URL for color versions. Nutritional pathology is reviewed by Roberts in Chapter 8. The content follows very closely the contribution of Roberts and Bullock to the second edition. Whether or not this reflects a paucity of recent research in this field during the last decade is left for specialists to decide. Chapter 9, by Hardy and Burrows, leads the reader through the applied aspects of diet formulation and manufacturing techniques. Expanded sections dealing with larval feeds and low-pollution feeds have been added. The treatment is thorough and the coverage of formulation methods and chemical and biological evaluations of feeds will be of particular value to new researchers in the field. Readers of this journal will welcome the prediction, that 'research and production of feeds formulated specifically for use in recirculation systems will be a growing sector of fish nutrition in the next decade'.

Chapter 10 is a review of adventitious toxins in feeds by Hendricks. Most of the material is familiar from the previous edition, with added discussion of the roles of recently encountered toxins e.g. the mycotoxin fumonisins. Chapter 11 is a short review by Piggot and Tucker of special feeds with a focus on some of the key major ingredients. Chapter 12 is a review by Gatlin of nutrition and fish health, and in particular the relationships between diet and immune function. This is new material added since the previous edition in what is now clearly an expanding field of investigation. Diet and fish husbandry, are reviewed by Lovell in Chapter 13. This follows the same format as in the previous edition, with details of the nutrient requirements and feeding practices for channel catfish, salmonids, tilapias and penaeid shrimp. The chapter brings into a practical perspective some of the previous material presented. A final

chapter contributed by the book's editors, Halver and Hardy, briefly summarizes patterns of nutrient flow and retention. An appendix lists examples of feed formulations, nutrient content of ingredients, examples of feeding charts and the scientific names of some aquaculture species.

The editors took on a formidable task in compiling a book of this scope and overall they have done it successfully. The book is comprehensive, there is little unnecessary repetition and the topics follow a logical sequence. Some short chapters may have best been combined with others e.g. information in the chapter dealing with special feeds could have been incorporated readily into the chapter dealing with diet formulation, which already covered most of the same topics. Isolated references to crustacean nutrition are found in three chapters. Readers may more usefully have been referred to specialized publications on this topic (e.g. D'Abramo et. al., 1997). The information presented is well referenced at the end of each chapter, although the omission of journal article titles in reference lists will frustrate some readers. Whilst new glossy covers have been added, the use of the same design and layout for the text and inclusion of many of the same figures and illustrations as in the previous edition do give the book a somewhat dated feel. There are few typographical or production errors for a book of this length although this reviewers copy had Table 13.4, which lists diet formulations for salmonids, duplicated at the expense of Table 13.12, which should have listed formulations for practical shrimp diets. The inclusion of a single color diagram (Fig. 12.1) is curious given that other flow charts were apparently submitted to the publishers as color slides where clarity could have benefited from color reproduction.

Since the publication of the previous edition of Fish Nutrition, numerous reviews, book chapters and both general and specialized books dealing with fish nutrition have been published. There is now a choice of texts available, with more accessible information, to support undergraduate courses in fish nutrition and aquaculture (e.g. De Silva and Anderson 1995, Lovell 1998, Guillaume et al. 2001). This new edition of Fish Nutrition will however continue its role as a leading source of reference for a wide readership, drawn from educators, researchers, aquaculturists and feed manufacturers.

References

DD'Abramo, L. R., Conklin, D. E. and Akiyama, D. M. (Eds.) **1997**. Crustacean Nutrition. 587 pages. World Aquaculture Society, Baton Rouge, LA, USA.

De Silva, S. S., Anderson, T. A. **1995**. Fish Nutrition in Aquaculture. 319 pages. Chapman and Hall, London, U.K.

Guillaume, J., Kaushik, S., Bergot, P. and Metailler, R. (Eds.) **2001**. Nutrition and Feeding of Fish and Crustaceans. 408 pages. Springer Verlag, Heidelberg, Germany.

Lovell, T. (Ed.) **1998**. Nutrition and Feeding of Fish, Second Edition. 267 pages. Kluwer Academic Publishers, Boston, MA, USA.

Public, Animal, and Environmental Aquaculture Health Issues

M.L. Jahncke, E.S. Garrett, A. Reilly, R.E. Martin, E. Cole (Eds.): John Wiley and Sons, Inc., New York, NY, USA. (2002). xix + 204 pp. ISBN: 0-471-38772-X US $48

Reviewed by: Bent Rønsholdt
Aalborg University
Sohngaardsholmsvej 57
DK-9000 Aalborg, Denmark

This book attempts to describe the aquaculture business in a broader, global perspective or, as the editors put it, using 'a holistic approach'. The book's focus is on aquaculture as a provider of produce for human consumption with concentration upon issues of public, animal and environmental health as conveyed by the title. Considering the complexity of the task, the general impression is that the subject is well covered and with a good organization of material.

The book contains six chapters, the first chapter: Status of World Fisheries and the Role of Aquaculture, providing a brief overview of world fisheries statistics and a short breakdown of world aquaculture production based on FAO statistics. It summarizes important issues associated with further development of global aquaculture that have been thematic for many scientific meetings on sustainable aquaculture in recent years (e.g., Stickney and McVey 2002, Baird et al. 1996, Reinertsen et al. 1995), and serves as an introduction to the remaining chapters.

Chapters 2 and 3 take their theme from the book title, separating the discussion from the viewpoint of non-industrialized and industrialized countries respectively. As the former chapter draws upon information generated in industrialized countries and since the latter's introduction discusses topics equally relevant to aquaculture management in non-industrialized countries (and vice versa in Chapter 2), this division seems rather artificial and the material in the two chapters has been repeated to

some extent. A look at the content page also shows the similarity in topics and categorization of health issues. Admittedly, in those categories the species and substances causing concern diverge, but this is probably more a consequence of differences in climatic and habitat environments as well as local practices. Furthermore, the general message from the book is the need for central planning and international consensus in addressing such issues in a similar manner. It is the actual application of a given concept that is bound to vary between nations and even locations, and it is here the major differences and challenges will appear (briefly mentioned in the conclusion in Chapter 4), also due to traditional, cultural and developmental differences. Therefore, a perhaps more interesting treatment of the subject would have been an investigation of the three major farming systems, extensive, semi-intensive and intensive in context with their public, animal and environmental issues, or vice versa, since the key point is the dependence on and interaction with the surroundings (broadly speaking) of these systems and their potential for control in various settings.

A short Chapter 4 introduces the HACCP concept as a superior inspection system to traditional end-product assessments as a means of assuring consumer safety. An overview of its current implementation in aquaculture is provided. The broadening of the HACCP concept from its original purpose to encompass control of aquatic animal diseases is discussed and an extension of the concept to manage aquaculture sites recommended. The interested reader should consult other sources for the actual content and methods of application of the concept in aquaculture, to which the reference list may serve as a starting point.

Chapter 5 deals with international organizations that provide guidelines and systems to regulate international trade, acquire consumer protection, and develop sustainable aquaculture. I found the overviews of the organizations having an impact on global aquaculture development, the importance of such bodies to serve as forums for global development of aquaculture, and as depositories of knowledge to be highly informative.

The final chapter summarizes the issues discussed earlier and provides the six indicators for future growth of global aquaculture listed by FAO. Consideration is also given to emerging obstacles to future expansion of the aquaculture industry; e.g., consumer acceptance of GMO's and ethical issues concerning use of animal protein as a feed source.

At first glance, this book seems to address itself to those involved in management and, in particular, risk assessment of aquaculture on a strategic level, e.g., managers, policy makers, public authorities, etc., but everybody with an interest in public, animal or environmental impact of aquaculture in a (global) community may benefit from viewing or relating their activities in such a perspective. For this purpose, the book is recommended as it provides coverage of the numerous and important issues within its topic in a systematic and comprehensible manner. Sections might have benefited with greater discussion upon how to cope with those same issues. The book illustrates very well the complexity of the aquaculture industry in a global setting and may serve as supplementary text or background information for students on a general introductory aquaculture or aquaculture management course. As the text is more descriptive than analytic, it does not provide answers, but may well assist in generating a checklist of issues that are or may be imposed on the industry. In-depth information on individual topics must be sought elsewhere, to which the references, and in particular those to publications from international organizations, given at the end of each chapter may prove useful.

International Journal of Recirculating Aquaculture

www.ijra.com

Instructions for Authors

The International Journal of Recirculating Aquaculture (IJRA) encourages authors to submit original research papers that present high-quality work on all aspects of recirculating aquaculture. Papers will be peer-reviewed and evaluated for scientific merit, relevance, and for their usefulness in promoting the advancement of recirculating aquaculture. Complete, coherent projects are preferred. Any related papers submitted together must be thoroughly cross-referenced.

All papers must be original, meaning that the data and the information presented must be the work of the authors, and cannot have been published elsewhere. Dual publication of a paper or data is possible only through the permission of the Editors of both journals. THERE ARE NO PAGE CHARGES for IJRA submissions.

MANUSCRIPT SUBMISSION

Manuscripts should be sent to Angela Correa, Managing Editor, *International Journal of Recirculating Aquaculture*. E-mail submission is preferred (ijra@vt.edu), but regular mail submissions are also accepted. Please send paper correspondence to:

27-D Food Science & Technology Building (0418)
Virginia Polytechnic Institute & State University
Blacksburg, VA 24061, USA

All manuscripts must be in English, and should be submitted both as a Microsoft Word document (.doc), and as an Adobe Acrobat document (.pdf). Mailed submissions should include the electronic files on a CD-ROM or USB 'thumb' drive. Please note that it is not possible to return media.

Each submission must include a cover letter stating that the paper contains original research and that the information has not been published elsewhere. Be sure to include the names of colleagues who have already reviewed the work presented.

IJRA has three categories for submission:

1. Articles, which are complete in-depth scientific studies;
2. Notes, which are short papers of limited scope, and
3. Book Reviews, which are summaries and opinions of recently-published aquaculture texts, 500-750 words in length.

Articles and notes will normally be critically reviewed by two or three experts, as well as by the Managing Editor. Book reviews will normally be critically reviewed by both the Executive Editor and the Managing Editor. Submissions may be returned without peer review in cases where the Managing Editor determines that they are inappropriate for the journal, of poor quality, poorly written, or fail to follow the journal's style format.

PREPARING the MANUSCRIPT

IJRA uses the latest editions of Webster's New International Dictionary and Webster's New Collegiate Dictionary for standard spellings and word definitions. All spellings should be in "American English." Foreign or science-related terminology that is unfamiliar and does not appear in these dictionaries will require an accompanying definition. All foreign language words should be italicized in the manuscript.

Acceptable scientific and common names of fishes are listed in A List of Common and Scientific Names of Fishes from the United States and Canada published by the American Fisheries Society. Scientific names should be italicized (e.g., *Antigonia aurorosea*). Common names may be used throughout each paper, but must be accompanied by a full scientific name when they first appear in a paper. The author must use the full common name, e.g., "yellow perch" not "perch."

Paper manuscripts must be submitted on paper 22 x 28 cm (8.5 x 11 inches) in size. Only one side of the paper should be used. Number each page sequentially and include the senior author's name next to the page number (e.g., Page 4, Flick) on each page. Single-space all typed material, including references. Type size in the body of the text should be no larger than Times New Roman 12pt font, and no smaller than Times New Roman 10pt font.

example: Times New Roman 12pt *Italic* **BOLD**
Times New Roman 10pt *Italic* **BOLD**

Subsection headings should be italicized in the manuscript. Spell out one-digit numbers, except when they are used with units of measure – e.g., six ponds, 7 days. Spell out any number that begins a sentence. When two numbers occur sequentially in the text, one of the numbers must be spelled out – e.g., In 2007, fifty fish were stocked. Use commas in numbers of 1,000 or more. Use the 24-hour clock to describe time – e.g., 1300, not 1:00 p.m. Always place a zero to the left of a decimal point if the number is less than one; this includes probability values – e.g., P = 0.05, not P = .05. All units of measurement must be reported via the metric system. Parts per million or milligrams per liter can be reported as either "ppm" or "mg/l," but not as "mg.l^{-1}."

Consult the current Edition of the ACS Style Guide (http://portal.acs.org) for detailed information regarding the use of standard international units (SIU) and measurements not mentioned here. Standard scientific abbreviations and symbols may be used without definition. If the use of a symbol could cause confusion, define the symbol the first time it is used.

Commonly used aquaculture jargon can be used without defining the term – e.g., fry, fingerling, hapa. Less commonly used jargon may be used, but terms should be defined the first time they are used. Jargon from fields outside of aquaculture should also be defined the first time they are used.

ORGANIZATION of MANUSCRIPTS

Articles and notes should include a title, names of authors and their addresses, abstract, introduction, materials and methods, results, discussion (or a combined results and discussion), conclusion (if needed), acknowledgements (if any), references, figures, and tables, in that order.

- **Title** – The title should accurately reflect the contents of the paper. Brief, concise titles are encouraged. The title page must include the name(s) of the author(s) and all titles and addresses. Use a separate page for the title page.
- **Keywords** – The author should place 5 or 6 key words within the abstract in bold text to be used for indexing.
- **Abstract** – The abstract should be a concise highlight of the results and conclusions. Methodology should not be abstracted unless it is necessary to explain the results or unless the paper describes a new technique. Abstracts should be less than 300 words. Use a separate page for the abstract.
- **Introduction** – The introduction should explain why the paper was written and why it is relevant. The introduction should condense the information or problems in the field that led the author to do the research. The introduction is not a complete literature review, and therefore only key references should be cited. The introduction should also contain a statement that describes the purpose or objective of the paper.
- **Materials and Methods** – The goal of this section is to clearly describe what was done so that others can repeat the experiment. If previously published papers described a technique that was used, citations can be used to prevent unnecessary repetition. If the technique or process was modified, the modification must be described so that others can repeat the process. Experimental designs can be explained by use of figures to help clarify what was done. If the experiment was a complicated one with many subparts, subsections may be used to describe each subpart.
- **Results** – This section describes the data. Proper use of tables and figures can simplify and help explain the results. Statistical analysis of data is necessary, unless differences are so obvious that statistical analysis is superfluous. Although probability values of 0.05 and 0.01 are traditionally used, each author is free to choose his or her own level of significance.
- **Discussion** – This section should interpret the results and compare the results from this experiment to those found in similar research. The section can also be used to speculate about the results, to provide reasons for the trends, and to suggest new ideas that can advance our understanding of aquaculture. This section should not be a

literature review.

- **Results and Discussion** -- The results and discussion sections can be combined. In most cases this leads to a better paper, because the integration of these sections leads to more meaningful interpretations of the data.
- **Conclusion** – This section should be used only when the results of an experiment lead to an unequivocal interpretation.
- **Acknowledgements** – This section should be used to thank organizations that provided monetary support for the research, as well as individuals who assisted in the research or preparation of the paper. This section includes manuscript number designations for those institutions that assign such numbers.
- **References** – Select references with great care. Unless your submission includes a literature review, there is no need to reference every paper written about a subject. Include only the most important ones.
- **Figures and Tables** – All tables and figures should follow the references. Figures go before tables. Number figures and tables to simplify referencing (e.g., Figure 1, or Figure 1a).

IN-TEXT CITATIONS --

Within the text, references are cited in one of two ways:

1. Fisher (2006) evaluated the role of laboratories. or—
2. The role of laboratories was evaluated (Mwanga 2006).

When there are three or more authors, list only the first in a citation and follow that with et al.:

Watanabe et al. (2009) reviewed tilapia mortality statistics.

When several citations are used in a single entry, they should be listed chronologically. If you are citing two papers that were written by the same author or sets of authors, the name only has to appear once per citation, and the name is followed by the years that designate the different papers; separate the years with commas. If you are citing two authors, you must separate the citations with a semicolon: (Schenkel 1999, 2006; Rusch 2004).

If the name of an institutional author is long, it can be abbreviated in a citation, so long as the full name appears in the references (e.g., National Institute for Food and Agriculture may be abbreviated as NIFA).

In the reference section, the reference list is strictly alphabetical by last name of the first author. If an author has written more than one paper, the following format should be followed: All single-authored papers go first, followed by coauthored papers, and then the papers that were written by three or more authors. If an author has written more than one single-authored paper, the papers are listed chronologically. If an author has written more than one co-authored paper, they are listed alphabetically by the second author's last name. If a tandem has authored two or more papers, the order is chronological. Papers written by the same group of three or more authors should be listed chronologically.

TABLES and FIGURES –
When creating a table, decide the purpose of the table and what points are important; then organize the table so that the main features can be easily understood. The table heading should provide sufficient information for the table to be understood as an entity. Keep footnotes to a minimum. Use the examples included with these instructions, as well as the ACS Style Guide for examples of acceptable tables. Each table goes on a separate page. Authors should endeavor to make tables fit on a single page. List any captions together with the table or figure. Although good quality photocopies of drawings or other artwork are acceptable when a manuscript is initially submitted for review, original artwork must accompany the final revised manuscript.

Graphs and original artwork must be printed with a laser printer or done in pen and ink on opaque white paper. Faint or illegible markings, or dot-matrix figures are not acceptable. Original artwork or high-contrast photographs are acceptable. Photographs should be on glossy paper. Do not glue pictures to cardboard. Color photographs will be changed to grayscale.

FINAL REVISED MANUSCRIPTS

Final manuscripts can be submitted electronically, either on disk or via e-mail, and should be submitted both as a Microsoft Word document (.doc). If necessary, one set of original artwork (drawings or photographs) can be submitted via regular mail.

REPRINTS -- The senior author and each junior author will receive two copies of the journal containing his or her article, along with a .pdf file of his or her article. These will be sent several weeks after the journal is published and in circulation. Questions regarding reprints should be e-mailed to ijra@vt.edu, or sent to IJRA, 27-D Food Science & Technology Building (0418), Virginia Tech, Blacksburg, VA 24061, USA.

COPYRIGHT & PERMISSIONS -- Questions regarding permission to reprint articles that have appeared in IJRA should be e-mailed to ijra@vt.edu, or sent to IJRA, 27-D Food Science & Technology Building (0418), Virginia Tech, Blacksburg, VA 24061, USA. For, please contact: IJRA, Copyright & Permissions, 119 Food Science & Technology Building, Virginia Tech, Blacksburg, VA 24061-0418, USA.

COPYRIGHT TRANSFER --If your manuscript is accepted for publication, copyright ownership must be officially transferred to IJRA. The Editor's acceptance letter will include the necessary form. The copyright transfer form must be signed by all authors and the original returned to the Editor at this time. Failure to return the copyright form in a timely fashion will result in delay in publication.