Reproduction and Selective Breeding of the Pacific Threadfin

General Information

Reporting Period June 1, 2002–September 30, 2004

Funding Level **$100,000**

Participants

Charles W. Laidley, Ph.D.
The Oceanic Institute

Robin J. Shields, Ph.D.
The Oceanic Institute

Objectives

1. Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.
2. Establish and maintain domesticated and selected Pacific threadfin broodstock lines.
3. Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.
4. Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance and indirect effects on survival, reproductive development, and generation time.
5. Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.
6. Initiate research on water reuse technology to protect selected broodstock lines from pathogen exposure and to decrease on-site water consumption.

Anticipated Benefits

Available estimates of heritable improvements in fish growth performance through genetic selection typically range from 10 to 23% per generation of selection amongst species examined to date. It is not unusual for these programs to require external support during early years due to the inherent time lags between program initiation and delivery of improved seedstock to farmers. However, the potential benefits to commercial aquaculture production in terms of improved growth and reduced production costs are significant. Most costs, with the exception of feeds, are tied to rates of production or growth. Thus the anticipated improvements in growth performance (i.e., 10 to 23% per round of selection) will reduce time to market on the order of 18 to 43 days and yield overall gains in farm profitability in the range of 6.5 to 15%. Based on modest gains in the range of 15% per generation of selection, the resulting improvement in industry efficiency would lead to increased profits of over $100,000 based on farm gates of approximately $1 million. These benefits will be further enhanced with industry expansion and with further rounds of selection.

Work Progress and Principal Accomplishments

Objective 1: Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups proved to be difficult, and therefore, eggs were obtained from larger group spawns.

The first objective was to examine methods of spawning Pacific threadfin broodstock in pairs or small groups to gain more control over the parental input to genetic lines, as opposed to current methods based on group spawning in larger broodstock tanks. During this project, we completed three experiments including (1) the use of luteinizing hormone-releasing hormone analog (LHRHa) implants to tank-spawn groups of 2, 4, or 8 broodstock, (2) the use of human chorionic gonadotropin (hCG) to induce tank spawning of paired broodstock, and experiments (3) and (4) in which hCG was used to induce final maturation and ovulation in conjunction with strip-spawning of females and artificial fertilization of eggs. Both hCG and LHRHa were successful in inducing egg-release from induced females, but spawns were not fertile. Experiments using hCG to strip-spawn females allowed the delineation of the time course for final oocyte maturation, but none of the females (total 10 tested) successfully ovulated, precluding completion of the strip-spawning protocol.

The high sensitivity of this species to stressors associated with the handling and manipulations necessary for strip-spawning procedures places too high a risk on valuable broodstock. Therefore, we have reverted to the original protocol of

obtaining eggs from larger group-spawns to ensure a supply of viable eggs and secure valuable lines of broodstock.

Objective 2: Establish and maintain domesticated and selected Pacific threadfin broodstock lines.

Growth-selected broodstock began spawning sporadically in May 2003, and the control broodstock followed in September. Both produced fertile spawns in July 2004.

Control and fast-growth fish were selected in August 2001 from a post-grow-out population of 604 fish at approximately six months of age at a mean weight/length of 358 g/25.6 cm. From this population, a 50-animal control group with mean weight/length of 378 g/25.6 cm and a 50-fish "select" group with mean weight/length of 516 g/28.9 cm were established (Table 1).

TABLE 1. *Survival, growth and reproductive development of control and select parental stocks of Pacific threadfin. Individual fish (identified by Passive Integrated Transponder [PIT] tags) were weighed, measured, and sexed at approximately 6, 12, and 16 months of age. Reproductive maturation is represented as the ratio of reproductively immature (I), male (M), and female (F) fish in each group.*

Age (mon.)	Date	Survival		Weight (g)		Length (cm)		Reprod. Matur. (I:M:F)	
		control	select	control	select	control	select	control	select
6	Aug 01	100%	100%	377	516	25.6	28.9	50:0:0	50:0:0
11	Jan 02	94%	100%	686	896	30.9	34.2	37:10:0	9:41:0
16	Jun 02	94%	98%	730	980	32.4	35.9	12:22:13	0:12:35
31	Sep 03	94%	84%	947	1173	35.6	38.2	11:22:14	1:10:31

January 2002 maturation checks (11 months of age) revealed that in addition to maintaining significant size difference between groups (31%), 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites), whereas 41 out of 50 growth-selected animals had entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (34%) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

By fall 2003, the growth-selected broodstock continued to maintain a distinct size advantage (24%) and a higher proportion of females than seen in control stocks. This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

The growth-selected group initiated monthly spawning activity in May 2003 (27 months of age), and the control group began sporadic spawning in September. Typical of first-spawning fish, spawning activity is highly sporadic, and fertility rates are quite low (<15%), impairing hatchery stocking. Multiple attempts were made in early 2004 to stimulate spawning activity through LHRHa implants, but spawns remained small and mostly infertile. In July 2004, both populations produced fertile spawns allowing hatchery stocking for the final phase of the project.

Objective 3: Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.

The July 2004 spawns from the growth-selected and control broodstock generated the seedstock needed for the growth performance evaluations.

The original work plan projected a one-year period for growth to sexual maturity and initiation of spawning. However, data obtained from this project suggests that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age, and only initiate spawning at around 2.5 years. Due to uncontrollable delays in broodstock spawning, a no-cost extension was granted to allow sufficient time for spawning and completion of the grow-out phases of the project.

In July 2004, both the domesticated control and growth-selected broodstock populations spawned, generating seedstock for growth performance evaluations. Although spawns were still small and of low fertility, they did generate sufficient numbers of fertile eggs to stock 1,000-L larval rearing tanks. Simultaneously, eggs from wild-collected broodstock were stocked in the production hatchery for use as a production control seedstock line. Larvae from all three lines were successfully reared using standard Pacific threadfin hatchery procedures, yielding 722 fingerlings from wild-collected broodstock (production controls), 716 fingerlings from control "non-selected" domesticated broodstock (domesticated controls), and 1,030 fingerlings from the growth-selected broodstock. An unexpected finding was that both domesticated lines, and in particular the growth-selected line, had lower rates of opercular deformities than fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production in support of the offshore cage industry (Figure 1).

Objective 4: Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time

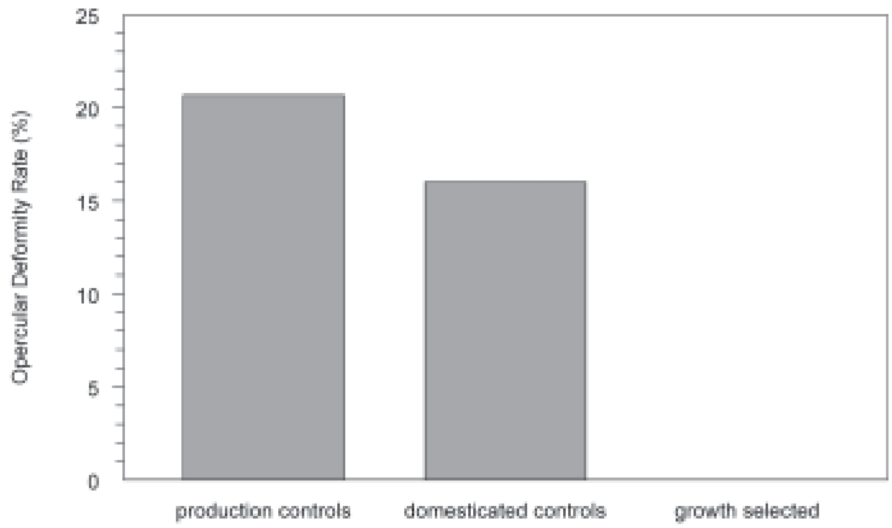


FIGURE 1. *Opercular deformity rates of fingerlings generated from domesticated control and growth-selected Pacific threadfin broodstock in comparison to that seen in fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production at the OI Production Hatchery (n=25).*

Groups of approximately 1,000 fingerlings derived from wild-collected broodstock (production controls), domesticated broodstock (domesticated controls), and growth-selected broodstock were stocked in 25-m^3 grow-out tanks and provided feed (Moore Clark Marine Grower Diet) to near-satiation levels using belt feeders throughout daylight hours. Samples of 50 fish are being weighed and measured once monthly to track growth and development. At three months of age when most juveniles had reached sufficient size (>10 cm) to survive the stressors associated with tagging, approximately 700 to 800 from each group were triple tagged, once with coded wire tags for specific identification and twice with visible elastomer implants (Figure 2) in the adipose tissue behind each eye for easy external identification. Note that juveniles less than 10 cm in length, known as runts, generally do not survive the tagging process and were culled from the populations. Interestingly, about 10% of the production and domesticated controls were runts while less than 1% of the growth-selected juveniles were runts.

Fish were then placed in a single 25-m^3 grow-out tank and will be split next month into two replicated 25-m^3 tanks with evenly mixed fish from each of the three treatment groups for subsequent growout of juvenile to market size. Fish will continue to be sampled monthly to track growth and reproductive development. Currently data have been collected up to three months of age just prior to tagging and stocking for the final grow-out phase (Figure 3). At present both the control and growth-selected lines are about 25% larger than fingerlings derived from wild-collected broodstock under large-scale fingerling production for the offshore cage industry. Note that although the growth-selected line left the hatchery at slightly lower sizes (length and weight) than controls, they have now caught up and slightly

FIGURE 2. *Photographs of the fingerling tagging process. The top left panel shows technical staff at OI in the process of tagging over 2,000 Pacific threadfin fingerlings for the CTSA-funded selective breeding project. Technical staff began the process by lightly anaesthetizing fingerlings and implanting individual coded wire tags (top right panel). The fish were then implanted with a highly visible fluorescent elastomer in the adipose tissue behind the eye, marking the treatment groups: green for production controls, red for domesticated controls, and yellow for growth-selected (see lower left panel). The bottom right panel shows fingerlings swimming in the recovery tanks immediately following implantation.*

surpassed (not significant) the domesticated control group. The next three months will determine if selection for growth is translated into significantly improved growth performance to market size.

Objective 5: Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

This portion of the project is scheduled post-grow-out and will be initiated subsequent to completion of the grow-out phase of control and select lines in approximately three months.

Objective 6: Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.

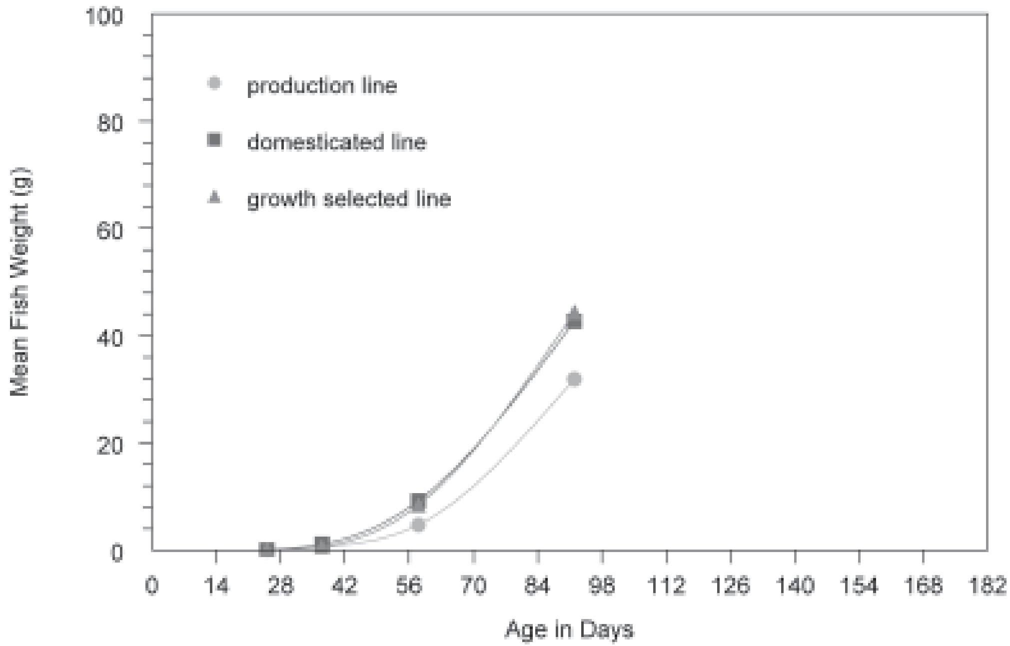


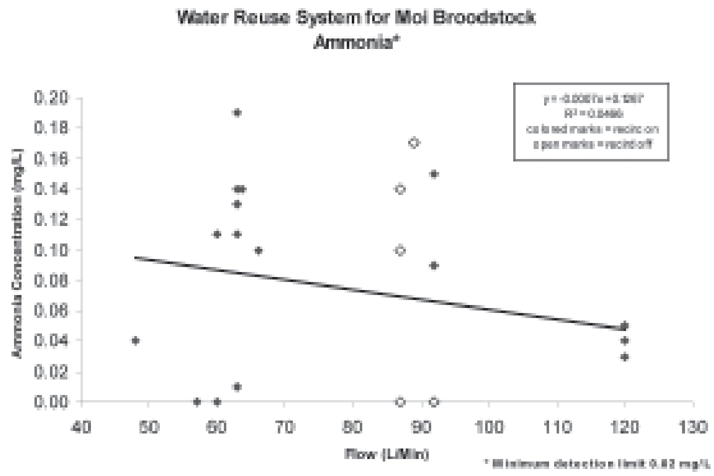
FIGURE 3. *Changes in body weight of production, domesticated, and growth-selected Pacific threadfin fingerlings through the first three months. Fish were sampled at day 25 upon harvest from hatchery to nursery I, at day 37 upon transfer from nursery I to nursery II, at day 58 upon transfer from nursery II to 25-m^3 outdoor grow-out tanks, and at day 92 during the tagging process. Fish will continue to be tracked until market size (about one pound) at around six to eight months of age.*

A low-cost water reuse system to maintain moi broodstock was installed in July 2004. The purpose of the system is to decrease water usage using various low-cost filters to maintain water quality. In addition to advantages in resource conservation, recirculation systems also can help mitigate against gas super-saturation and the high dissolved CO_2 levels / low pH levels (~pH 7.5) characteristic of water derived from saltwater wells typical of the Pacific Islands. A lower flow of water into the tank would result in a longer residence time, thus helping degas some of the CO_2 and increase pH.

To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

1) 120 L/min, reuse system on
2) 90 L/min, reuse system on
3) 60 L/min, reuse system on
4) 90 L/min, reuse system off

There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).



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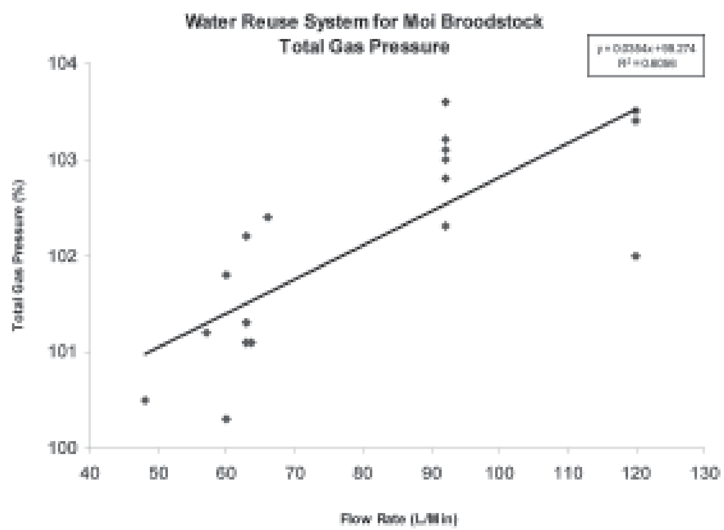

FIGURE 5. *Total gas pressure (% saturation) of water from a moi broodstock tank with water flow ranging from 45–120 L/min.*

Ammonia was negatively correlated (non-significant) to flow, although ammonia concentrations did not exceed 0.20 ppm (Figure 4). Total gas pressure was positively correlated with flow rate ($r^2 = 0.61$) (Figure 5), although comparisons could not be made between system on and system off due to malfunction of the gas saturometer during the experiment.

In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

required water use by about 50% before detrimentally affecting fish behavior and food consumption, but the additional system did not improve or alleviate gas saturation or low water pH. Based on these results we would suggest the need for more powerful recirculation equipment with special emphasis on equipment designed to degas water to lower total gas saturation, lower dissolved CO_2, and increase system pH.

Work Planned

1. Continue to maintain Pacific threadfin broodstock lines.
2. Complete juvenile grow-out to determine direct effects of selection on growth performance and indirect effects on survival. Longer-term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (3+ years vs. 1 year) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival and dressing percentage.
5. Provide final project report to CTSA and publish findings in CTSA *Regional Notes*.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants and retail markets and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both onshore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C.W. 2004. Marine finfish culture technology at the Oceanic Institute. Page 325 *in* Aquaculture 2004: Book of Abstracts. March 2–5, 2004, Honolulu, Hawaii. World Aquaculture Society, Baton Rouge, Louisiana, USA.

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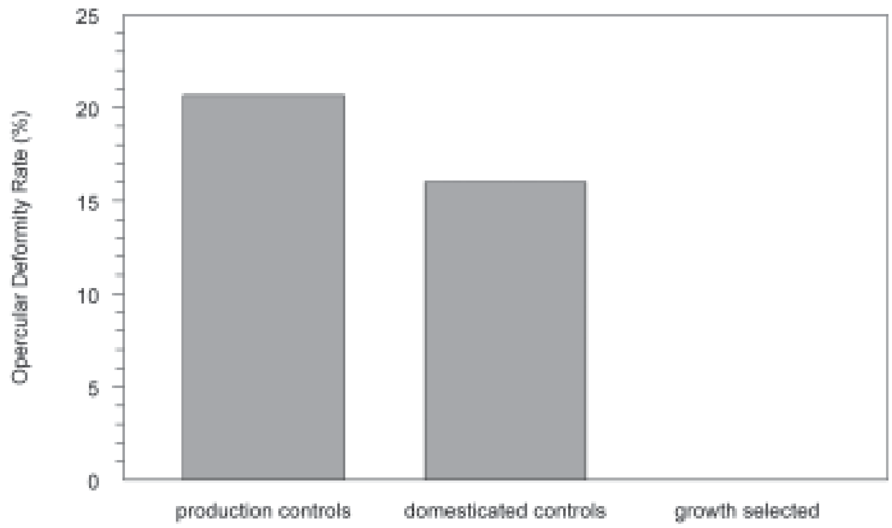

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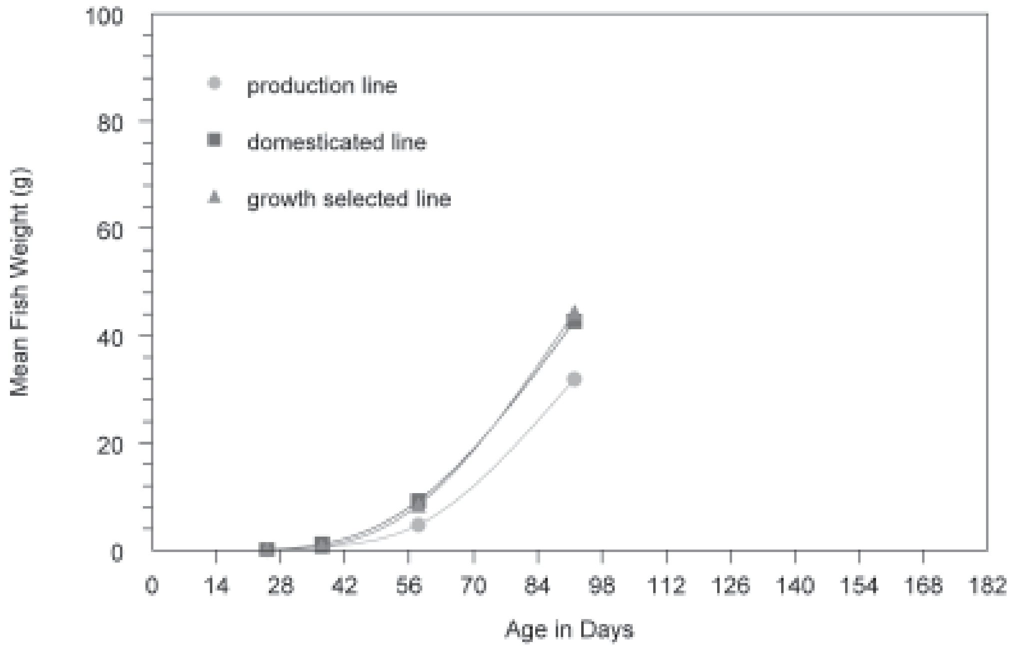

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To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

1) 120 L/min, reuse system on
2) 90 L/min, reuse system on
3) 60 L/min, reuse system on
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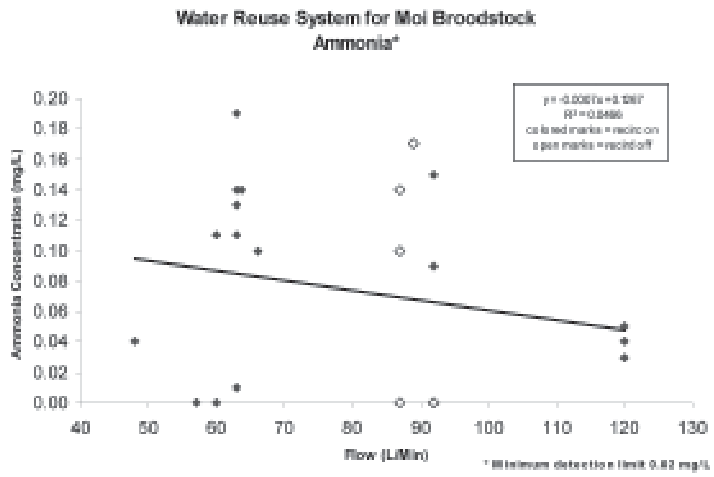
There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).



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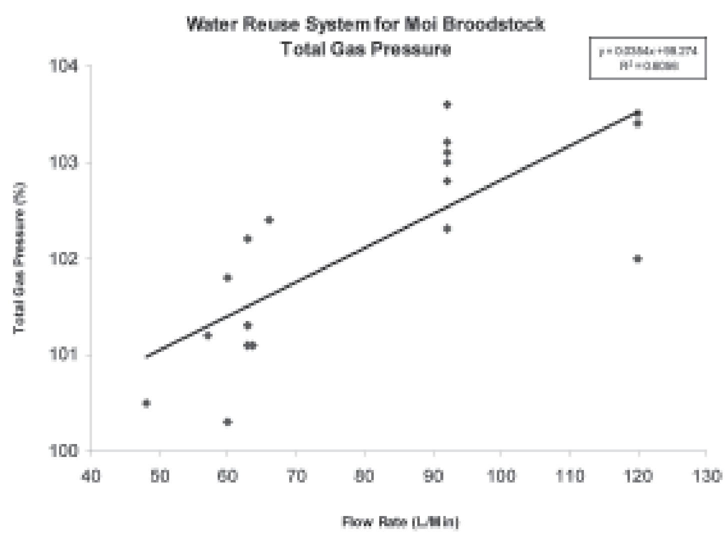

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In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

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Work Planned

1. Continue to maintain Pacific threadfin broodstock lines.
2. Complete juvenile grow-out to determine direct effects of selection on growth performance and indirect effects on survival. Longer-term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (3+ years vs. 1 year) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival and dressing percentage.
5. Provide final project report to CTSA and publish findings in CTSA *Regional Notes*.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants and retail markets and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both onshore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C.W. 2004. Marine finfish culture technology at the Oceanic Institute. Page 325 *in* Aquaculture 2004: Book of Abstracts. March 2–5, 2004, Honolulu, Hawaii. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Reproduction and Selective Breeding of the Pacific Threadfin

General Information

Reporting Period	June 1, 2002–September 30, 2004
Funding Level	**$100,000**
Participants	**Charles W. Laidley**, Ph.D. The Oceanic Institute Robin J. Shields, Ph.D. The Oceanic Institute

Objectives

1. Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.
2. Establish and maintain domesticated and selected Pacific threadfin broodstock lines.
3. Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.
4. Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance and indirect effects on survival, reproductive development, and generation time.
5. Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.
6. Initiate research on water reuse technology to protect selected broodstock lines from pathogen exposure and to decrease on-site water consumption.

Anticipated Benefits

Available estimates of heritable improvements in fish growth performance through genetic selection typically range from 10 to 23% per generation of selection amongst species examined to date. It is not unusual for these programs to require external support during early years due to the inherent time lags between program initiation and delivery of improved seedstock to farmers. However, the potential benefits to commercial aquaculture production in terms of improved growth and reduced production costs are significant. Most costs, with the exception of feeds, are tied to rates of production or growth. Thus the anticipated improvements in growth performance (i.e., 10 to 23% per round of selection) will reduce time to market on the order of 18 to 43 days and yield overall gains in farm profitability in the range of 6.5 to 15%. Based on modest gains in the range of 15% per generation of selection, the resulting improvement in industry efficiency would lead to increased profits of over $100,000 based on farm gates of approximately $1 million. These benefits will be further enhanced with industry expansion and with further rounds of selection.

Work Progress and Principal Accomplishments

Objective 1: Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups proved to be difficult, and therefore, eggs were obtained from larger group spawns.

The first objective was to examine methods of spawning Pacific threadfin broodstock in pairs or small groups to gain more control over the parental input to genetic lines, as opposed to current methods based on group spawning in larger broodstock tanks. During this project, we completed three experiments including (1) the use of luteinizing hormone-releasing hormone analog (LHRHa) implants to tank-spawn groups of 2, 4, or 8 broodstock, (2) the use of human chorionic gonadotropin (hCG) to induce tank spawning of paired broodstock, and experiments (3) and (4) in which hCG was used to induce final maturation and ovulation in conjunction with strip-spawning of females and artificial fertilization of eggs. Both hCG and LHRHa were successful in inducing egg-release from induced females, but spawns were not fertile. Experiments using hCG to strip-spawn females allowed the delineation of the time course for final oocyte maturation, but none of the females (total 10 tested) successfully ovulated, precluding completion of the strip-spawning protocol.

The high sensitivity of this species to stressors associated with the handling and manipulations necessary for strip-spawning procedures places too high a risk on valuable broodstock. Therefore, we have reverted to the original protocol of

obtaining eggs from larger group-spawns to ensure a supply of viable eggs and secure valuable lines of broodstock.

Objective 2: Establish and maintain domesticated and selected Pacific threadfin broodstock lines.

Growth-selected broodstock began spawning sporadically in May 2003, and the control broodstock followed in September. Both produced fertile spawns in July 2004.

Control and fast-growth fish were selected in August 2001 from a post-grow-out population of 604 fish at approximately six months of age at a mean weight/length of 358 g/25.6 cm. From this population, a 50-animal control group with mean weight/length of 378 g/25.6 cm and a 50-fish "select" group with mean weight/length of 516 g/28.9 cm were established (Table 1).

TABLE 1. *Survival, growth and reproductive development of control and select parental stocks of Pacific threadfin. Individual fish (identified by Passive Integrated Transponder [PIT] tags) were weighed, measured, and sexed at approximately 6, 12, and 16 months of age. Reproductive maturation is represented as the ratio of reproductively immature (I), male (M), and female (F) fish in each group.*

Age (mon.)	Date	Survival		Weight (g)		Length (cm)		Reprod. Matur. (I:M:F)	
		control	select	control	select	control	select	control	select
6	Aug 01	100%	100%	377	516	25.6	28.9	50:0:0	50:0:0
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16	Jun 02	94%	98%	730	980	32.4	35.9	12:22:13	0:12:35
31	Sep 03	94%	84%	947	1173	35.6	38.2	11:22:14	1:10:31

January 2002 maturation checks (11 months of age) revealed that in addition to maintaining significant size difference between groups (31%), 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites), whereas 41 out of 50 growth-selected animals had entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (34%) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

By fall 2003, the growth-selected broodstock continued to maintain a distinct size advantage (24%) and a higher proportion of females than seen in control stocks. This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

The growth-selected group initiated monthly spawning activity in May 2003 (27 months of age), and the control group began sporadic spawning in September. Typical of first-spawning fish, spawning activity is highly sporadic, and fertility rates are quite low (<15%), impairing hatchery stocking. Multiple attempts were made in early 2004 to stimulate spawning activity through LHRHa implants, but spawns remained small and mostly infertile. In July 2004, both populations produced fertile spawns allowing hatchery stocking for the final phase of the project.

Objective 3: Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.

The July 2004 spawns from the growth-selected and control broodstock generated the seedstock needed for the growth performance evaluations.

The original work plan projected a one-year period for growth to sexual maturity and initiation of spawning. However, data obtained from this project suggests that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age, and only initiate spawning at around 2.5 years. Due to uncontrollable delays in broodstock spawning, a no-cost extension was granted to allow sufficient time for spawning and completion of the grow-out phases of the project.

In July 2004, both the domesticated control and growth-selected broodstock populations spawned, generating seedstock for growth performance evaluations. Although spawns were still small and of low fertility, they did generate sufficient numbers of fertile eggs to stock 1,000-L larval rearing tanks. Simultaneously, eggs from wild-collected broodstock were stocked in the production hatchery for use as a production control seedstock line. Larvae from all three lines were successfully reared using standard Pacific threadfin hatchery procedures, yielding 722 fingerlings from wild-collected broodstock (production controls), 716 fingerlings from control "non-selected" domesticated broodstock (domesticated controls), and 1,030 fingerlings from the growth-selected broodstock. An unexpected finding was that both domesticated lines, and in particular the growth-selected line, had lower rates of opercular deformities than fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production in support of the offshore cage industry (Figure 1).

Objective 4: Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time

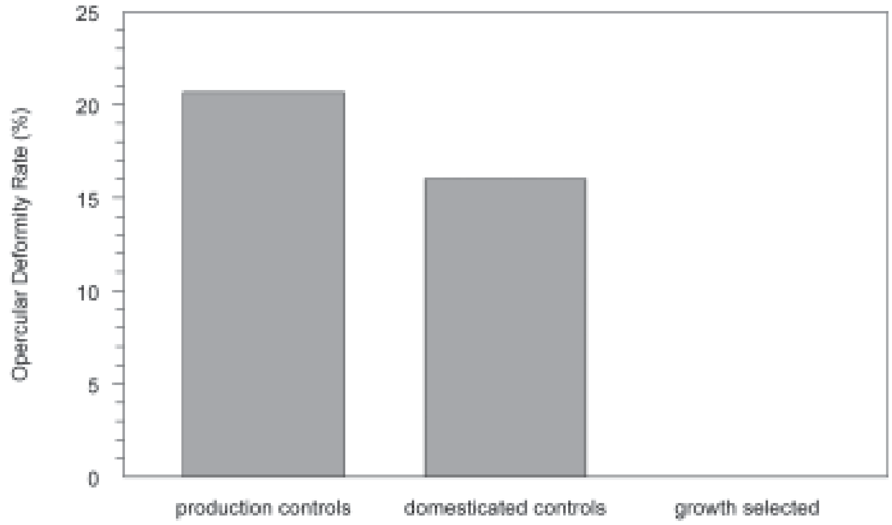

FIGURE 1. *Opercular deformity rates of fingerlings generated from domesticated control and growth-selected Pacific threadfin broodstock in comparison to that seen in fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production at the OI Production Hatchery (n=25).*

Groups of approximately 1,000 fingerlings derived from wild-collected broodstock (production controls), domesticated broodstock (domesticated controls), and growth-selected broodstock were stocked in 25-m^3 grow-out tanks and provided feed (Moore Clark Marine Grower Diet) to near-satiation levels using belt feeders throughout daylight hours. Samples of 50 fish are being weighed and measured once monthly to track growth and development. At three months of age when most juveniles had reached sufficient size (>10 cm) to survive the stressors associated with tagging, approximately 700 to 800 from each group were triple tagged, once with coded wire tags for specific identification and twice with visible elastomer implants (Figure 2) in the adipose tissue behind each eye for easy external identification. Note that juveniles less than 10 cm in length, known as runts, generally do not survive the tagging process and were culled from the populations. Interestingly, about 10% of the production and domesticated controls were runts while less than 1% of the growth-selected juveniles were runts.

Fish were then placed in a single 25-m^3 grow-out tank and will be split next month into two replicated 25-m^3 tanks with evenly mixed fish from each of the three treatment groups for subsequent growout of juvenile to market size. Fish will continue to be sampled monthly to track growth and reproductive development. Currently data have been collected up to three months of age just prior to tagging and stocking for the final grow-out phase (Figure 3). At present both the control and growth-selected lines are about 25% larger than fingerlings derived from wild-collected broodstock under large-scale fingerling production for the offshore cage industry. Note that although the growth-selected line left the hatchery at slightly lower sizes (length and weight) than controls, they have now caught up and slightly

FIGURE 2. *Photographs of the fingerling tagging process. The top left panel shows technical staff at OI in the process of tagging over 2,000 Pacific threadfin fingerlings for the CTSA-funded selective breeding project. Technical staff began the process by lightly anaesthetizing fingerlings and implanting individual coded wire tags (top right panel). The fish were then implanted with a highly visible fluorescent elastomer in the adipose tissue behind the eye, marking the treatment groups: green for production controls, red for domesticated controls, and yellow for growth-selected (see lower left panel). The bottom right panel shows fingerlings swimming in the recovery tanks immediately following implantation.*

surpassed (not significant) the domesticated control group. The next three months will determine if selection for growth is translated into significantly improved growth performance to market size.

Objective 5: Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

This portion of the project is scheduled post-grow-out and will be initiated subsequent to completion of the grow-out phase of control and select lines in approximately three months.

Objective 6: Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.

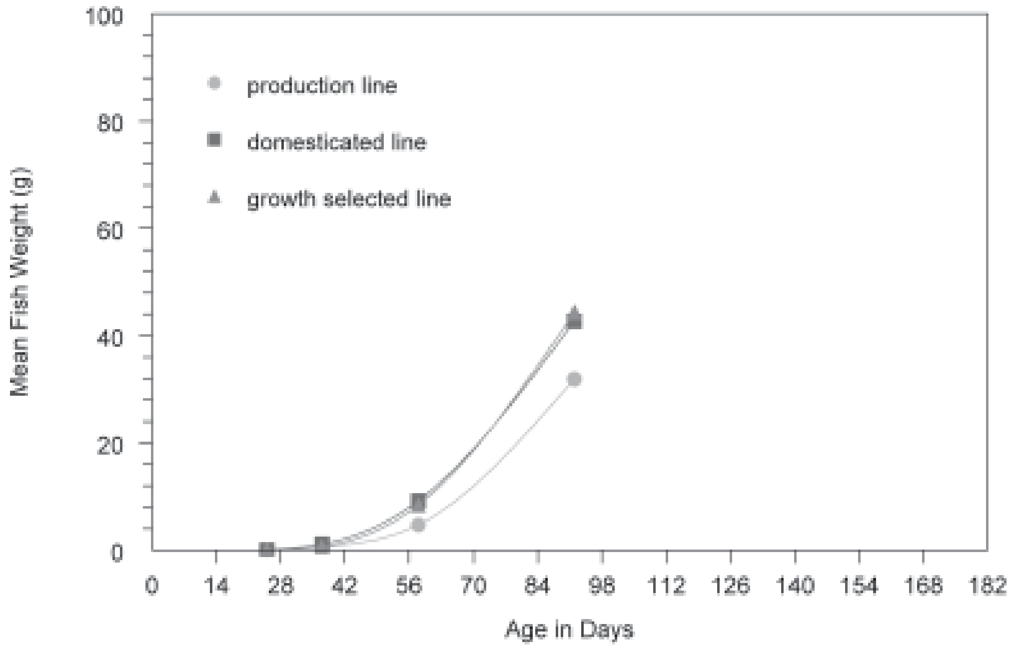

FIGURE 3. *Changes in body weight of production, domesticated, and growth-selected Pacific threadfin fingerlings through the first three months. Fish were sampled at day 25 upon harvest from hatchery to nursery I, at day 37 upon transfer from nursery I to nursery II, at day 58 upon transfer from nursery II to 25-m^3 outdoor grow-out tanks, and at day 92 during the tagging process. Fish will continue to be tracked until market size (about one pound) at around six to eight months of age.*

A low-cost water reuse system to maintain moi broodstock was installed in July 2004. The purpose of the system is to decrease water usage using various low-cost filters to maintain water quality. In addition to advantages in resource conservation, recirculation systems also can help mitigate against gas super-saturation and the high dissolved CO_2 levels / low pH levels (~pH 7.5) characteristic of water derived from saltwater wells typical of the Pacific Islands. A lower flow of water into the tank would result in a longer residence time, thus helping degas some of the CO_2 and increase pH.

To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

1) 120 L/min, reuse system on
2) 90 L/min, reuse system on
3) 60 L/min, reuse system on
4) 90 L/min, reuse system off

There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).

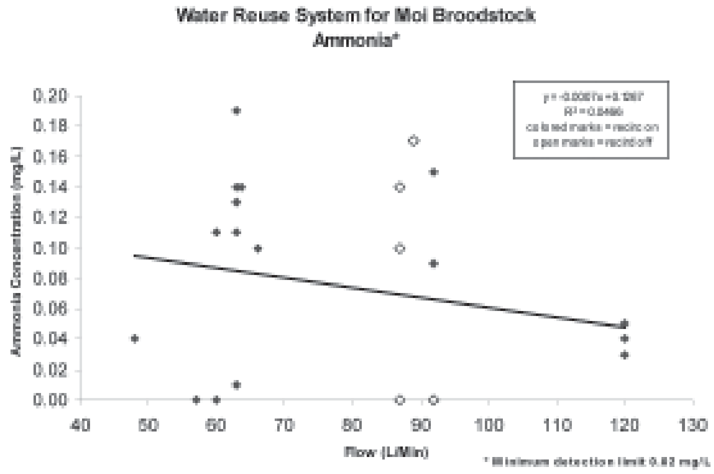

FIGURE 4. *Ammonia concentrations (mg/L) of water from a moi broodstock tank outfitted with a low-cost water reuse system.*

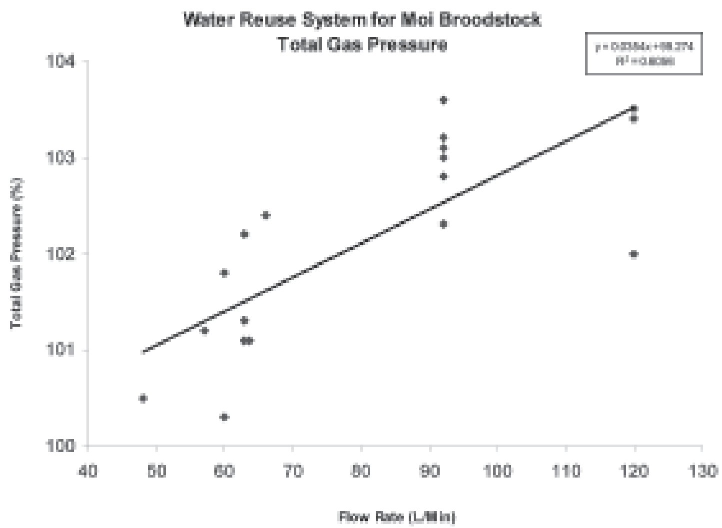

FIGURE 5. *Total gas pressure (% saturation) of water from a moi broodstock tank with water flow ranging from 45–120 L/min.*

Ammonia was negatively correlated (non-significant) to flow, although ammonia concentrations did not exceed 0.20 ppm (Figure 4). Total gas pressure was positively correlated with flow rate ($r^2 = 0.61$) (Figure 5), although comparisons could not be made between system on and system off due to malfunction of the gas saturometer during the experiment.

In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

required water use by about 50% before detrimentally affecting fish behavior and food consumption, but the additional system did not improve or alleviate gas saturation or low water pH. Based on these results we would suggest the need for more powerful recirculation equipment with special emphasis on equipment designed to degas water to lower total gas saturation, lower dissolved CO_2, and increase system pH.

Work Planned

1. Continue to maintain Pacific threadfin broodstock lines.
2. Complete juvenile grow-out to determine direct effects of selection on growth performance and indirect effects on survival. Longer-term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (3+ years vs. 1 year) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival and dressing percentage.
5. Provide final project report to CTSA and publish findings in CTSA *Regional Notes*.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants and retail markets and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both onshore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

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Funding Level **$100,000**

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Anticipated Benefits

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Work Progress and Principal Accomplishments

Objective 1: Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups proved to be difficult, and therefore, eggs were obtained from larger group spawns.

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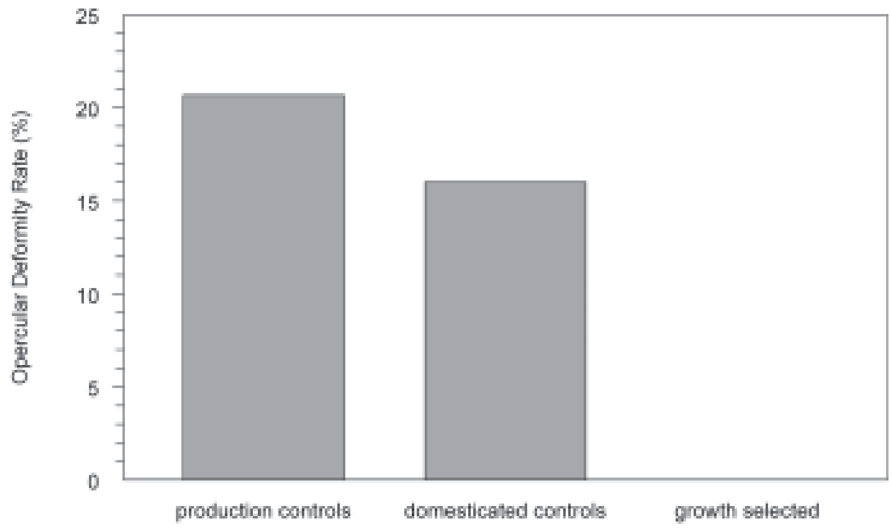
Objective 4: Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time



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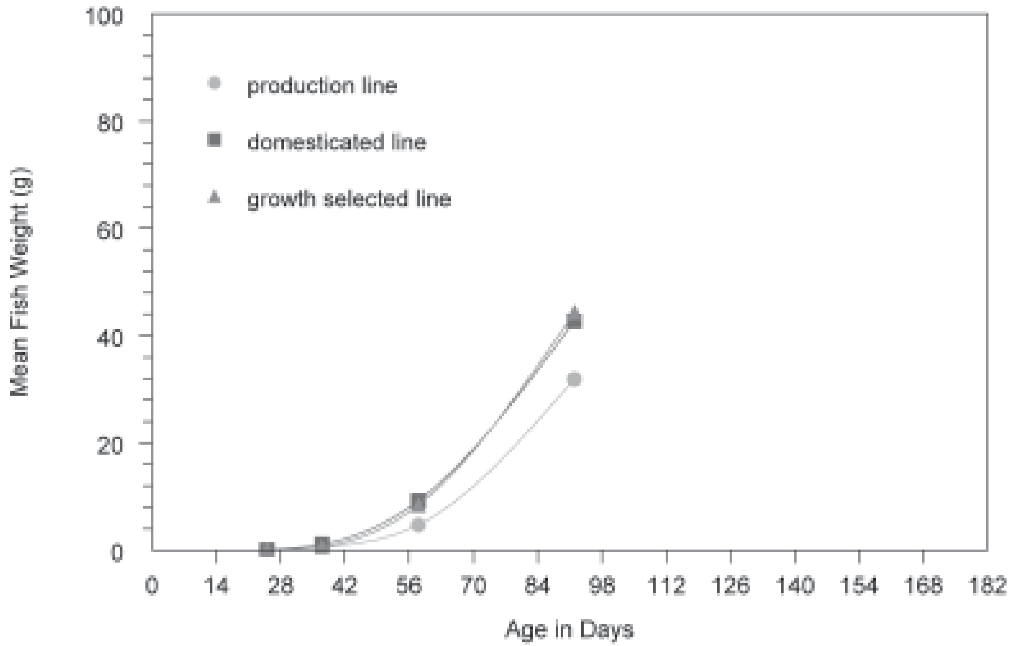
Objective 6: Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.



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To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

1) 120 L/min, reuse system on
2) 90 L/min, reuse system on
3) 60 L/min, reuse system on
4) 90 L/min, reuse system off

There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).

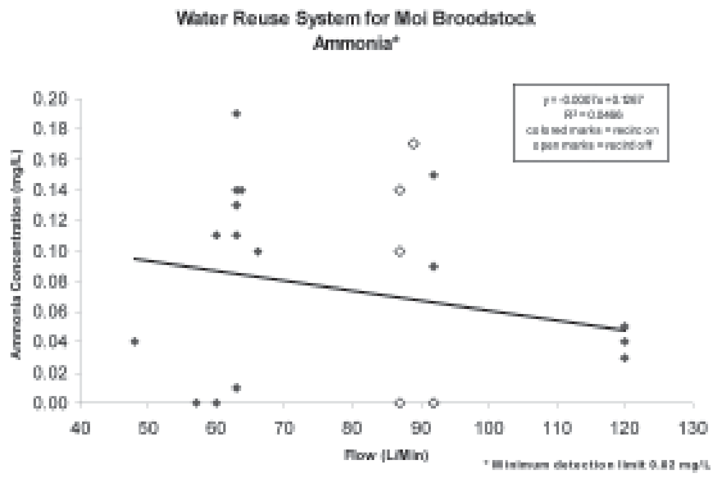

FIGURE 4. *Ammonia concentrations (mg/L) of water from a moi broodstock tank outfitted with a low-cost water reuse system.*

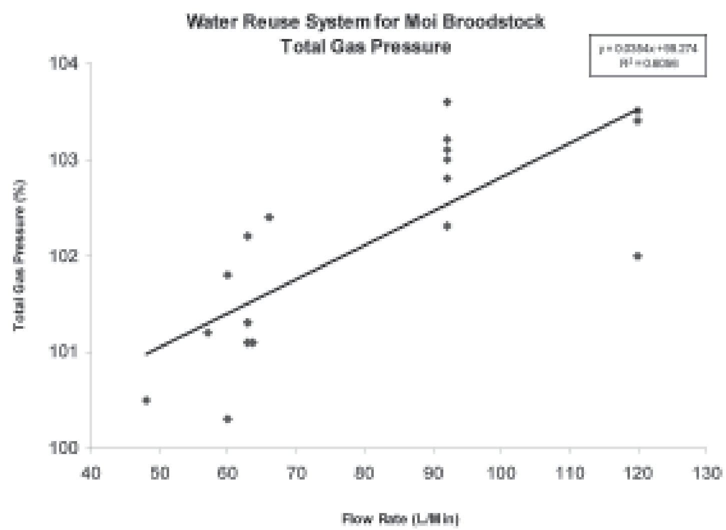

FIGURE 5. *Total gas pressure (% saturation) of water from a moi broodstock tank with water flow ranging from 45–120 L/min.*

Ammonia was negatively correlated (non-significant) to flow, although ammonia concentrations did not exceed 0.20 ppm (Figure 4). Total gas pressure was positively correlated with flow rate ($r^2 = 0.61$) (Figure 5), although comparisons could not be made between system on and system off due to malfunction of the gas saturometer during the experiment.

In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

required water use by about 50% before detrimentally affecting fish behavior and food consumption, but the additional system did not improve or alleviate gas saturation or low water pH. Based on these results we would suggest the need for more powerful recirculation equipment with special emphasis on equipment designed to degas water to lower total gas saturation, lower dissolved CO_2, and increase system pH.

Work Planned

1. Continue to maintain Pacific threadfin broodstock lines.
2. Complete juvenile grow-out to determine direct effects of selection on growth performance and indirect effects on survival. Longer-term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (3+ years vs. 1 year) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival and dressing percentage.
5. Provide final project report to CTSA and publish findings in CTSA *Regional Notes*.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants and retail markets and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both onshore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C.W. 2004. Marine finfish culture technology at the Oceanic Institute. Page 325 *in* Aquaculture 2004: Book of Abstracts. March 2–5, 2004, Honolulu, Hawaii. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Reproduction and Selective Breeding of the Pacific Threadfin

General Information

Reporting Period June 1, 2002–September 30, 2004

Funding Level **$100,000**

Participants **Charles W. Laidley**, Ph.D.
The Oceanic Institute

Robin J. Shields, Ph.D.
The Oceanic Institute

Objectives

1. Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.
2. Establish and maintain domesticated and selected Pacific threadfin broodstock lines.
3. Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.
4. Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance and indirect effects on survival, reproductive development, and generation time.
5. Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.
6. Initiate research on water reuse technology to protect selected broodstock lines from pathogen exposure and to decrease on-site water consumption.

Anticipated Benefits

Available estimates of heritable improvements in fish growth performance through genetic selection typically range from 10 to 23% per generation of selection amongst species examined to date. It is not unusual for these programs to require external support during early years due to the inherent time lags between program initiation and delivery of improved seedstock to farmers. However, the potential benefits to commercial aquaculture production in terms of improved growth and reduced production costs are significant. Most costs, with the exception of feeds, are tied to rates of production or growth. Thus the anticipated improvements in growth performance (i.e., 10 to 23% per round of selection) will reduce time to market on the order of 18 to 43 days and yield overall gains in farm profitability in the range of 6.5 to 15%. Based on modest gains in the range of 15% per generation of selection, the resulting improvement in industry efficiency would lead to increased profits of over $100,000 based on farm gates of approximately $1 million. These benefits will be further enhanced with industry expansion and with further rounds of selection.

Work Progress and Principal Accomplishments

Objective 1: Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups proved to be difficult, and therefore, eggs were obtained from larger group spawns.

The first objective was to examine methods of spawning Pacific threadfin broodstock in pairs or small groups to gain more control over the parental input to genetic lines, as opposed to current methods based on group spawning in larger broodstock tanks. During this project, we completed three experiments including (1) the use of luteinizing hormone-releasing hormone analog (LHRHa) implants to tank-spawn groups of 2, 4, or 8 broodstock, (2) the use of human chorionic gonadotropin (hCG) to induce tank spawning of paired broodstock, and experiments (3) and (4) in which hCG was used to induce final maturation and ovulation in conjunction with strip-spawning of females and artificial fertilization of eggs. Both hCG and LHRHa were successful in inducing egg-release from induced females, but spawns were not fertile. Experiments using hCG to strip-spawn females allowed the delineation of the time course for final oocyte maturation, but none of the females (total 10 tested) successfully ovulated, precluding completion of the strip-spawning protocol.

The high sensitivity of this species to stressors associated with the handling and manipulations necessary for strip-spawning procedures places too high a risk on valuable broodstock. Therefore, we have reverted to the original protocol of

obtaining eggs from larger group-spawns to ensure a supply of viable eggs and secure valuable lines of broodstock.

Objective 2: Establish and maintain domesticated and selected Pacific threadfin broodstock lines.

Growth-selected broodstock began spawning sporadically in May 2003, and the control broodstock followed in September. Both produced fertile spawns in July 2004.

Control and fast-growth fish were selected in August 2001 from a post-grow-out population of 604 fish at approximately six months of age at a mean weight/length of 358 g/25.6 cm. From this population, a 50-animal control group with mean weight/length of 378 g/25.6 cm and a 50-fish "select" group with mean weight/length of 516 g/28.9 cm were established (Table 1).

TABLE 1. *Survival, growth and reproductive development of control and select parental stocks of Pacific threadfin. Individual fish (identified by Passive Integrated Transponder [PIT] tags) were weighed, measured, and sexed at approximately 6, 12, and 16 months of age. Reproductive maturation is represented as the ratio of reproductively immature (I), male (M), and female (F) fish in each group.*

Age (mon.)	Date	Survival		Weight (g)		Length (cm)		Reprod. Matur. (I:M:F)	
		control	select	control	select	control	select	control	select
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31	Sep 03	94%	84%	947	1173	35.6	38.2	11:22:14	1:10:31

January 2002 maturation checks (11 months of age) revealed that in addition to maintaining significant size difference between groups (31%), 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites), whereas 41 out of 50 growth-selected animals had entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (34%) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

By fall 2003, the growth-selected broodstock continued to maintain a distinct size advantage (24%) and a higher proportion of females than seen in control stocks. This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

The growth-selected group initiated monthly spawning activity in May 2003 (27 months of age), and the control group began sporadic spawning in September. Typical of first-spawning fish, spawning activity is highly sporadic, and fertility rates are quite low (<15%), impairing hatchery stocking. Multiple attempts were made in early 2004 to stimulate spawning activity through LHRHa implants, but spawns remained small and mostly infertile. In July 2004, both populations produced fertile spawns allowing hatchery stocking for the final phase of the project.

Objective 3: Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.

The July 2004 spawns from the growth-selected and control broodstock generated the seedstock needed for the growth performance evaluations.

The original work plan projected a one-year period for growth to sexual maturity and initiation of spawning. However, data obtained from this project suggests that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age, and only initiate spawning at around 2.5 years. Due to uncontrollable delays in broodstock spawning, a no-cost extension was granted to allow sufficient time for spawning and completion of the grow-out phases of the project.

In July 2004, both the domesticated control and growth-selected broodstock populations spawned, generating seedstock for growth performance evaluations. Although spawns were still small and of low fertility, they did generate sufficient numbers of fertile eggs to stock 1,000-L larval rearing tanks. Simultaneously, eggs from wild-collected broodstock were stocked in the production hatchery for use as a production control seedstock line. Larvae from all three lines were successfully reared using standard Pacific threadfin hatchery procedures, yielding 722 fingerlings from wild-collected broodstock (production controls), 716 fingerlings from control "non-selected" domesticated broodstock (domesticated controls), and 1,030 fingerlings from the growth-selected broodstock. An unexpected finding was that both domesticated lines, and in particular the growth-selected line, had lower rates of opercular deformities than fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production in support of the offshore cage industry (Figure 1).

Objective 4: Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time

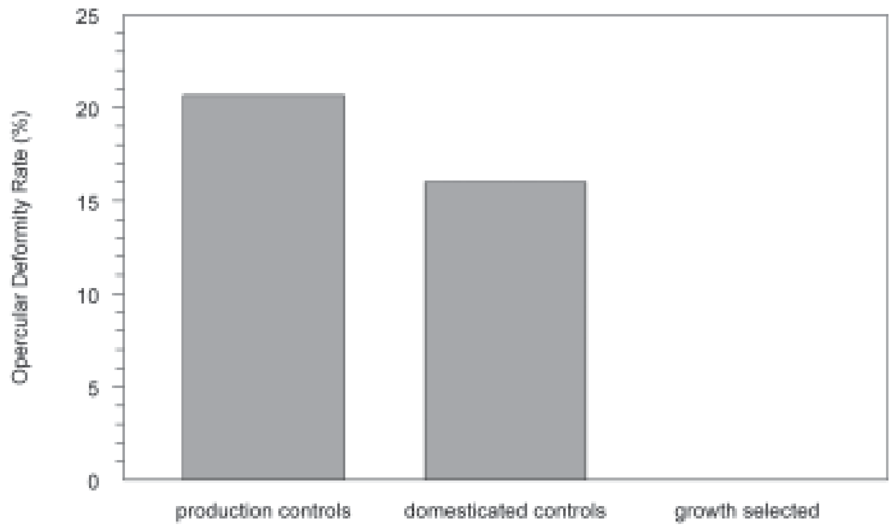


FIGURE 1. *Opercular deformity rates of fingerlings generated from domesticated control and growth-selected Pacific threadfin broodstock in comparison to that seen in fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production at the OI Production Hatchery (n=25).*

Groups of approximately 1,000 fingerlings derived from wild-collected broodstock (production controls), domesticated broodstock (domesticated controls), and growth-selected broodstock were stocked in 25-m^3 grow-out tanks and provided feed (Moore Clark Marine Grower Diet) to near-satiation levels using belt feeders throughout daylight hours. Samples of 50 fish are being weighed and measured once monthly to track growth and development. At three months of age when most juveniles had reached sufficient size (>10 cm) to survive the stressors associated with tagging, approximately 700 to 800 from each group were triple tagged, once with coded wire tags for specific identification and twice with visible elastomer implants (Figure 2) in the adipose tissue behind each eye for easy external identification. Note that juveniles less than 10 cm in length, known as runts, generally do not survive the tagging process and were culled from the populations. Interestingly, about 10% of the production and domesticated controls were runts while less than 1% of the growth-selected juveniles were runts.

Fish were then placed in a single 25-m^3 grow-out tank and will be split next month into two replicated 25-m^3 tanks with evenly mixed fish from each of the three treatment groups for subsequent growout of juvenile to market size. Fish will continue to be sampled monthly to track growth and reproductive development. Currently data have been collected up to three months of age just prior to tagging and stocking for the final grow-out phase (Figure 3). At present both the control and growth-selected lines are about 25% larger than fingerlings derived from wild-collected broodstock under large-scale fingerling production for the offshore cage industry. Note that although the growth-selected line left the hatchery at slightly lower sizes (length and weight) than controls, they have now caught up and slightly

FIGURE 2. *Photographs of the fingerling tagging process. The top left panel shows technical staff at OI in the process of tagging over 2,000 Pacific threadfin fingerlings for the CTSA-funded selective breeding project. Technical staff began the process by lightly anaesthetizing fingerlings and implanting individual coded wire tags (top right panel). The fish were then implanted with a highly visible fluorescent elastomer in the adipose tissue behind the eye, marking the treatment groups: green for production controls, red for domesticated controls, and yellow for growth-selected (see lower left panel). The bottom right panel shows fingerlings swimming in the recovery tanks immediately following implantation.*

surpassed (not significant) the domesticated control group. The next three months will determine if selection for growth is translated into significantly improved growth performance to market size.

Objective 5: Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

This portion of the project is scheduled post-grow-out and will be initiated subsequent to completion of the grow-out phase of control and select lines in approximately three months.

Objective 6: Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.

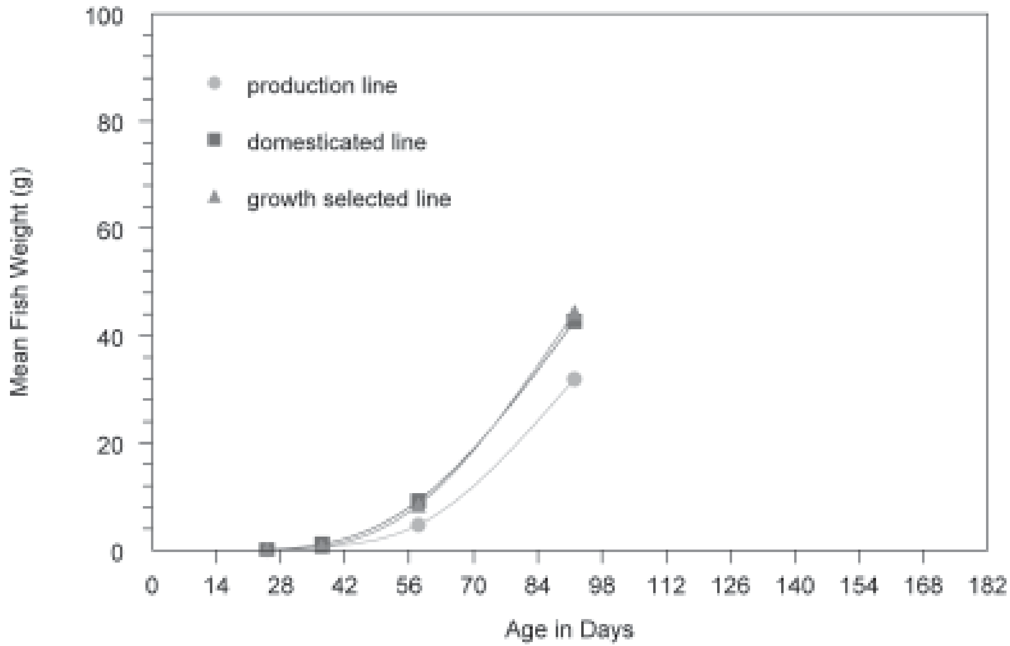


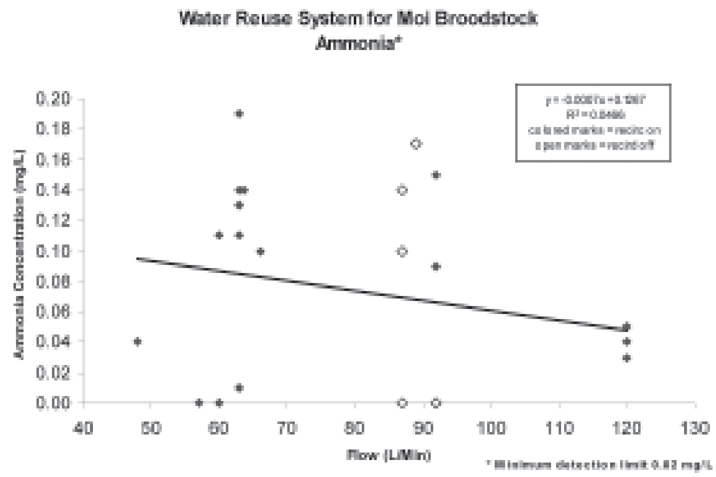
FIGURE 3. *Changes in body weight of production, domesticated, and growth-selected Pacific threadfin fingerlings through the first three months. Fish were sampled at day 25 upon harvest from hatchery to nursery I, at day 37 upon transfer from nursery I to nursery II, at day 58 upon transfer from nursery II to 25-m^3 outdoor grow-out tanks, and at day 92 during the tagging process. Fish will continue to be tracked until market size (about one pound) at around six to eight months of age.*

A low-cost water reuse system to maintain moi broodstock was installed in July 2004. The purpose of the system is to decrease water usage using various low-cost filters to maintain water quality. In addition to advantages in resource conservation, recirculation systems also can help mitigate against gas super-saturation and the high dissolved CO_2 levels / low pH levels (~pH 7.5) characteristic of water derived from saltwater wells typical of the Pacific Islands. A lower flow of water into the tank would result in a longer residence time, thus helping degas some of the CO_2 and increase pH.

To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

1) 120 L/min, reuse system on
2) 90 L/min, reuse system on
3) 60 L/min, reuse system on
4) 90 L/min, reuse system off

There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).



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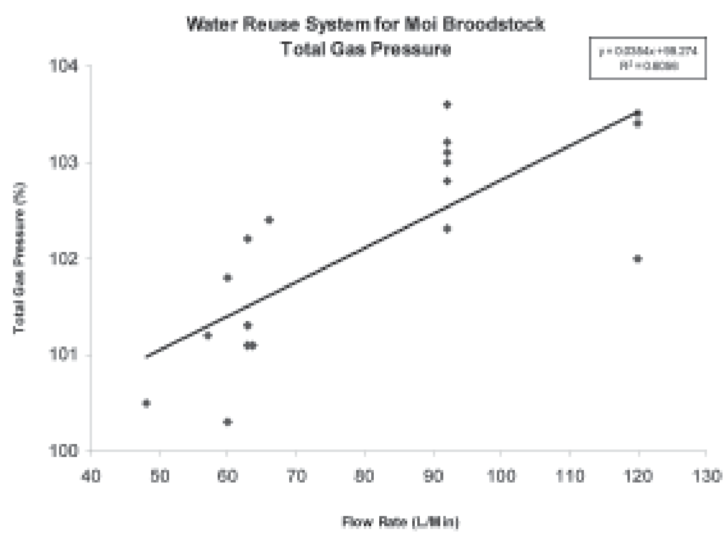

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Ammonia was negatively correlated (non-significant) to flow, although ammonia concentrations did not exceed 0.20 ppm (Figure 4). Total gas pressure was positively correlated with flow rate ($r^2 = 0.61$) (Figure 5), although comparisons could not be made between system on and system off due to malfunction of the gas saturometer during the experiment.

In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

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1. Continue to maintain Pacific threadfin broodstock lines.
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Funding Level	**$100,000**
Participants	**Charles W. Laidley**, Ph.D. The Oceanic Institute Robin J. Shields, Ph.D. The Oceanic Institute

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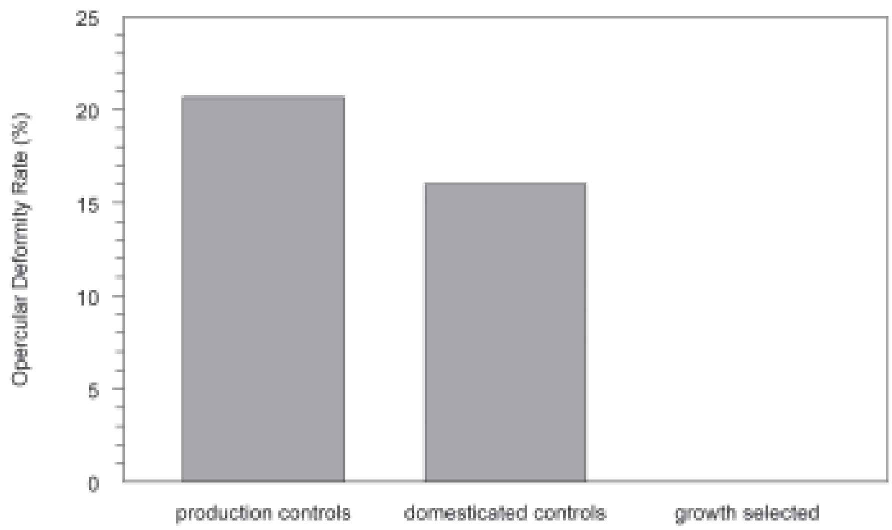

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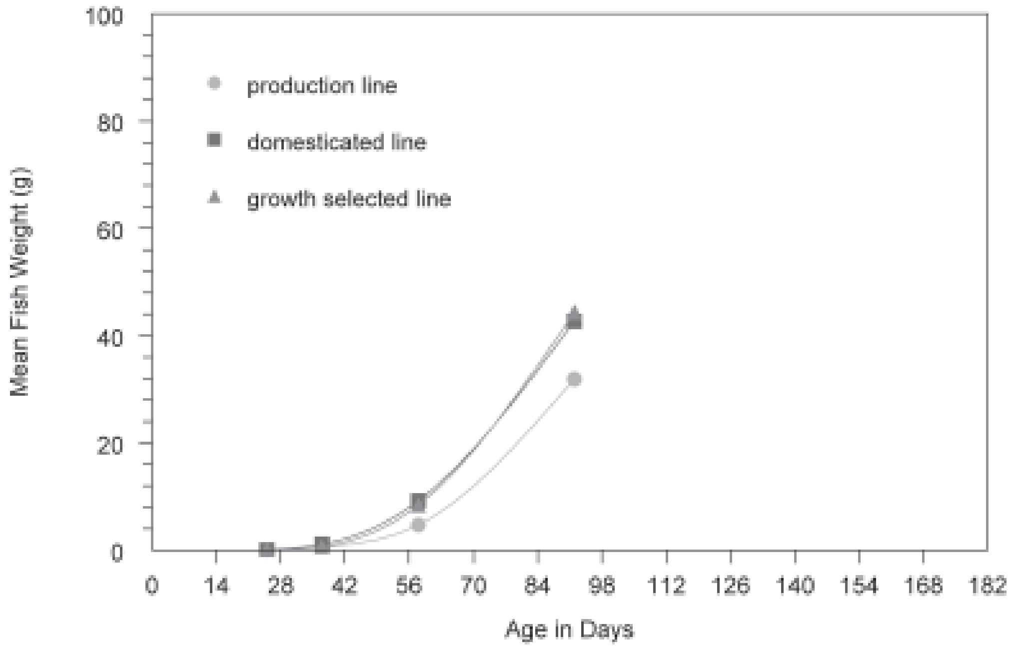

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To evaluate system performance, the system was tested under a series of operational conditions for a period of at least four days that included:

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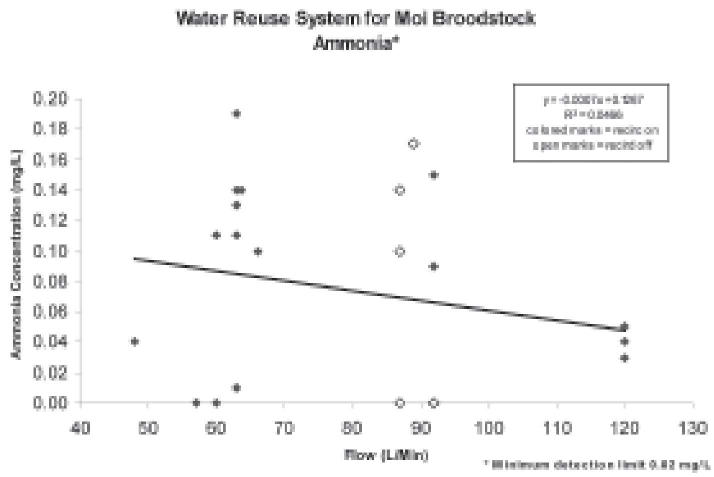
There was no correlation among flow rates with the reuse system on or off in terms of pH (7.37 to 7.77), temperature (26.74 to 27.46), or salinity (33.03 to 33.15).



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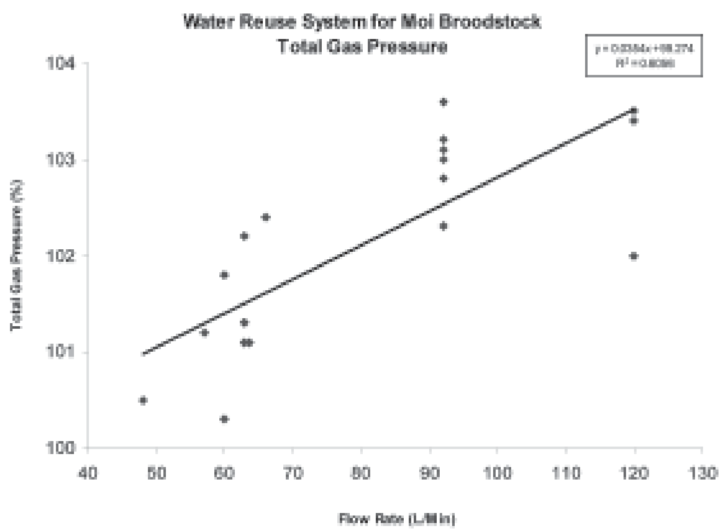


FIGURE 5. *Total gas pressure (% saturation) of water from a moi broodstock tank with water flow ranging from 45–120 L/min.*

Ammonia was negatively correlated (non-significant) to flow, although ammonia concentrations did not exceed 0.20 ppm (Figure 4). Total gas pressure was positively correlated with flow rate ($r^2 = 0.61$) (Figure 5), although comparisons could not be made between system on and system off due to malfunction of the gas saturometer during the experiment.

In summary, it appears that the low-cost (~$5,000) water reuse system installed for maintaining moi broodstock (approximately ninety 2- to 3-kg fish) did reduce

required water use by about 50% before detrimentally affecting fish behavior and food consumption, but the additional system did not improve or alleviate gas saturation or low water pH. Based on these results we would suggest the need for more powerful recirculation equipment with special emphasis on equipment designed to degas water to lower total gas saturation, lower dissolved CO_2, and increase system pH.

Work Planned

1. Continue to maintain Pacific threadfin broodstock lines.
2. Complete juvenile grow-out to determine direct effects of selection on growth performance and indirect effects on survival. Longer-term examination of reproductive development and generation time may not be possible without long-term grant extension due to the much longer than expected (3+ years vs. 1 year) generation time for the species.
4. Gain estimate of heritability for growth and indirect effects on survival and dressing percentage.
5. Provide final project report to CTSA and publish findings in CTSA *Regional Notes*.

Impacts

The aquaculture development of the Pacific threadfin is gaining substantial momentum in Hawaii with the appearance of captive farmed product in local restaurants and retail markets and sales to both mainland and international markets. Recent adoption of cage culture technologies based on the joint OI/SeaGrant Hawaii Offshore Aquaculture Research Project (HOARP) has further intensified production capability in the sector. CTSA-funded research has provided the cornerstone for this growing industry to date, and expected developments under current project funding will further assist in securing requisite fingerling supplies to meet the needs of both onshore and offshore production. Current efforts to enhance aquaculture performance through genetic selection will provide new opportunities to increase industry efficiency through improved growth, reduced generation time, and greater resistance to stress and disease.

Publications in Print, Manuscripts, and Papers Presented

Laidley, C.W. 2004. Marine finfish culture technology at the Oceanic Institute. Page 325 *in* Aquaculture 2004: Book of Abstracts. March 2–5, 2004, Honolulu, Hawaii. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Reproduction and Selective Breeding of the Pacific Threadfin

General Information

Reporting Period	June 1, 2002–September 30, 2004
Funding Level	**$100,000**
Participants	**Charles W. Laidley**, Ph.D. The Oceanic Institute Robin J. Shields, Ph.D. The Oceanic Institute

Objectives

1. Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.
2. Establish and maintain domesticated and selected Pacific threadfin broodstock lines.
3. Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.
4. Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance and indirect effects on survival, reproductive development, and generation time.
5. Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.
6. Initiate research on water reuse technology to protect selected broodstock lines from pathogen exposure and to decrease on-site water consumption.

Anticipated Benefits

Available estimates of heritable improvements in fish growth performance through genetic selection typically range from 10 to 23% per generation of selection amongst species examined to date. It is not unusual for these programs to require external support during early years due to the inherent time lags between program initiation and delivery of improved seedstock to farmers. However, the potential benefits to commercial aquaculture production in terms of improved growth and reduced production costs are significant. Most costs, with the exception of feeds, are tied to rates of production or growth. Thus the anticipated improvements in growth performance (i.e., 10 to 23% per round of selection) will reduce time to market on the order of 18 to 43 days and yield overall gains in farm profitability in the range of 6.5 to 15%. Based on modest gains in the range of 15% per generation of selection, the resulting improvement in industry efficiency would lead to increased profits of over $100,000 based on farm gates of approximately $1 million. These benefits will be further enhanced with industry expansion and with further rounds of selection.

Work Progress and Principal Accomplishments

Objective 1: Complete methods development for pair spawning of Pacific threadfin for application to the genetic selection efforts.

Strip-spawning Pacific threadfin in pairs or small groups proved to be difficult, and therefore, eggs were obtained from larger group spawns.

The first objective was to examine methods of spawning Pacific threadfin broodstock in pairs or small groups to gain more control over the parental input to genetic lines, as opposed to current methods based on group spawning in larger broodstock tanks. During this project, we completed three experiments including (1) the use of luteinizing hormone-releasing hormone analog (LHRHa) implants to tank-spawn groups of 2, 4, or 8 broodstock, (2) the use of human chorionic gonadotropin (hCG) to induce tank spawning of paired broodstock, and experiments (3) and (4) in which hCG was used to induce final maturation and ovulation in conjunction with strip-spawning of females and artificial fertilization of eggs. Both hCG and LHRHa were successful in inducing egg-release from induced females, but spawns were not fertile. Experiments using hCG to strip-spawn females allowed the delineation of the time course for final oocyte maturation, but none of the females (total 10 tested) successfully ovulated, precluding completion of the strip-spawning protocol.

The high sensitivity of this species to stressors associated with the handling and manipulations necessary for strip-spawning procedures places too high a risk on valuable broodstock. Therefore, we have reverted to the original protocol of

obtaining eggs from larger group-spawns to ensure a supply of viable eggs and secure valuable lines of broodstock.

Objective 2: Establish and maintain domesticated and selected Pacific threadfin broodstock lines.

Growth-selected broodstock began spawning sporadically in May 2003, and the control broodstock followed in September. Both produced fertile spawns in July 2004.

Control and fast-growth fish were selected in August 2001 from a post-grow-out population of 604 fish at approximately six months of age at a mean weight/length of 358 g/25.6 cm. From this population, a 50-animal control group with mean weight/length of 378 g/25.6 cm and a 50-fish "select" group with mean weight/length of 516 g/28.9 cm were established (Table 1).

TABLE 1. *Survival, growth and reproductive development of control and select parental stocks of Pacific threadfin. Individual fish (identified by Passive Integrated Transponder [PIT] tags) were weighed, measured, and sexed at approximately 6, 12, and 16 months of age. Reproductive maturation is represented as the ratio of reproductively immature (I), male (M), and female (F) fish in each group.*

Age (mon.)	Date	Survival		Weight (g)		Length (cm)		Reprod. Matur. (I:M:F)	
		control	select	control	select	control	select	control	select
6	Aug 01	100%	100%	377	516	25.6	28.9	50:0:0	50:0:0
11	Jan 02	94%	100%	686	896	30.9	34.2	37:10:0	9:41:0
16	Jun 02	94%	98%	730	980	32.4	35.9	12:22:13	0:12:35
31	Sep 03	94%	84%	947	1173	35.6	38.2	11:22:14	1:10:31

January 2002 maturation checks (11 months of age) revealed that in addition to maintaining significant size difference between groups (31%), 10 out of 47 control animals had reached the male stage of sexual maturity (Pacific threadfin are protandrous hermaphrodites), whereas 41 out of 50 growth-selected animals had entered the male phase of sexual maturation.

June maturation checks of these same stocks again revealed a significant size difference (34%) and the appearance of the first female animals. Interestingly, the majority of the select group has rapidly proceeded to the female stage of development with 12 males and 35 females while the control group was slower to develop reproductively with 12 fish remaining immature, 22 as males, and 13 advancing to the female stage.

By fall 2003, the growth-selected broodstock continued to maintain a distinct size advantage (24%) and a higher proportion of females than seen in control stocks. This data also suggests that size, rather than environmental or behavioral conditions appear to be more important in determining the timing of sexual development and sex change in captive stocks of Pacific threadfin.

The growth-selected group initiated monthly spawning activity in May 2003 (27 months of age), and the control group began sporadic spawning in September. Typical of first-spawning fish, spawning activity is highly sporadic, and fertility rates are quite low (<15%), impairing hatchery stocking. Multiple attempts were made in early 2004 to stimulate spawning activity through LHRHa implants, but spawns remained small and mostly infertile. In July 2004, both populations produced fertile spawns allowing hatchery stocking for the final phase of the project.

Objective 3: Conduct controlled spawning of select broodstock lines to generate select seedstock for growth performance evaluation.

The July 2004 spawns from the growth-selected and control broodstock generated the seedstock needed for the growth performance evaluations.

The original work plan projected a one-year period for growth to sexual maturity and initiation of spawning. However, data obtained from this project suggests that portions of the Pacific threadfin broodstock population reach the female stage of development between 11 and 16 months of age, and only initiate spawning at around 2.5 years. Due to uncontrollable delays in broodstock spawning, a no-cost extension was granted to allow sufficient time for spawning and completion of the grow-out phases of the project.

In July 2004, both the domesticated control and growth-selected broodstock populations spawned, generating seedstock for growth performance evaluations. Although spawns were still small and of low fertility, they did generate sufficient numbers of fertile eggs to stock 1,000-L larval rearing tanks. Simultaneously, eggs from wild-collected broodstock were stocked in the production hatchery for use as a production control seedstock line. Larvae from all three lines were successfully reared using standard Pacific threadfin hatchery procedures, yielding 722 fingerlings from wild-collected broodstock (production controls), 716 fingerlings from control “non-selected” domesticated broodstock (domesticated controls), and 1,030 fingerlings from the growth-selected broodstock. An unexpected finding was that both domesticated lines, and in particular the growth-selected line, had lower rates of opercular deformities than fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production in support of the offshore cage industry (Figure 1).

Objective 4: Complete life cycle of growth-selected and control lines of Pacific threadfin and determine direct effects of selection on growth performance, and indirect effects on survival, reproductive development, and generation time

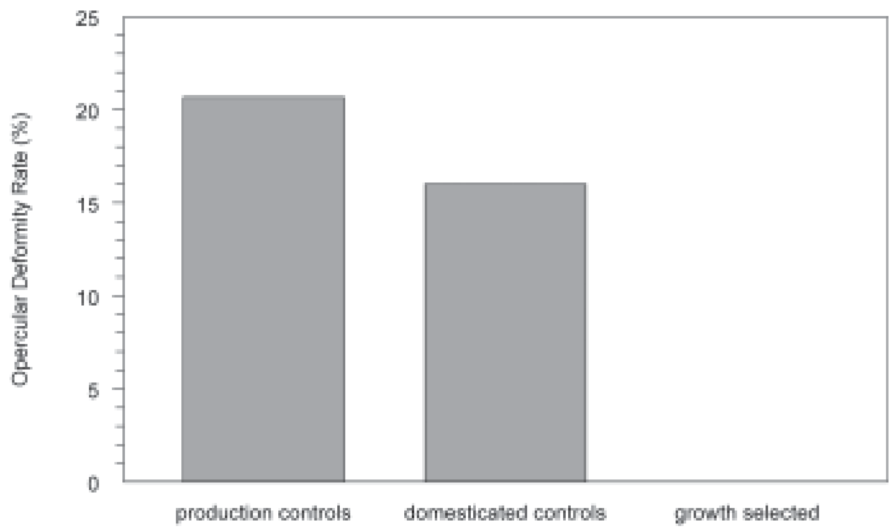


FIGURE 1. *Opercular deformity rates of fingerlings generated from domesticated control and growth-selected Pacific threadfin broodstock in comparison to that seen in fingerlings generated from wild-collected broodstock currently used for large-scale fingerling production at the OI Production Hatchery (n=25).*

Groups of approximately 1,000 fingerlings derived from wild-collected broodstock (production controls), domesticated broodstock (domesticated controls), and growth-selected broodstock were stocked in 25-m^3 grow-out tanks and provided feed (Moore Clark Marine Grower Diet) to near-satiation levels using belt feeders throughout daylight hours. Samples of 50 fish are being weighed and measured once monthly to track growth and development. At three months of age when most juveniles had reached sufficient size (>10 cm) to survive the stressors associated with tagging, approximately 700 to 800 from each group were triple tagged, once with coded wire tags for specific identification and twice with visible elastomer implants (Figure 2) in the adipose tissue behind each eye for easy external identification. Note that juveniles less than 10 cm in length, known as runts, generally do not survive the tagging process and were culled from the populations. Interestingly, about 10% of the production and domesticated controls were runts while less than 1% of the growth-selected juveniles were runts.

Fish were then placed in a single 25-m^3 grow-out tank and will be split next month into two replicated 25-m^3 tanks with evenly mixed fish from each of the three treatment groups for subsequent growout of juvenile to market size. Fish will continue to be sampled monthly to track growth and reproductive development. Currently data have been collected up to three months of age just prior to tagging and stocking for the final grow-out phase (Figure 3). At present both the control and growth-selected lines are about 25% larger than fingerlings derived from wild-collected broodstock under large-scale fingerling production for the offshore cage industry. Note that although the growth-selected line left the hatchery at slightly lower sizes (length and weight) than controls, they have now caught up and slightly

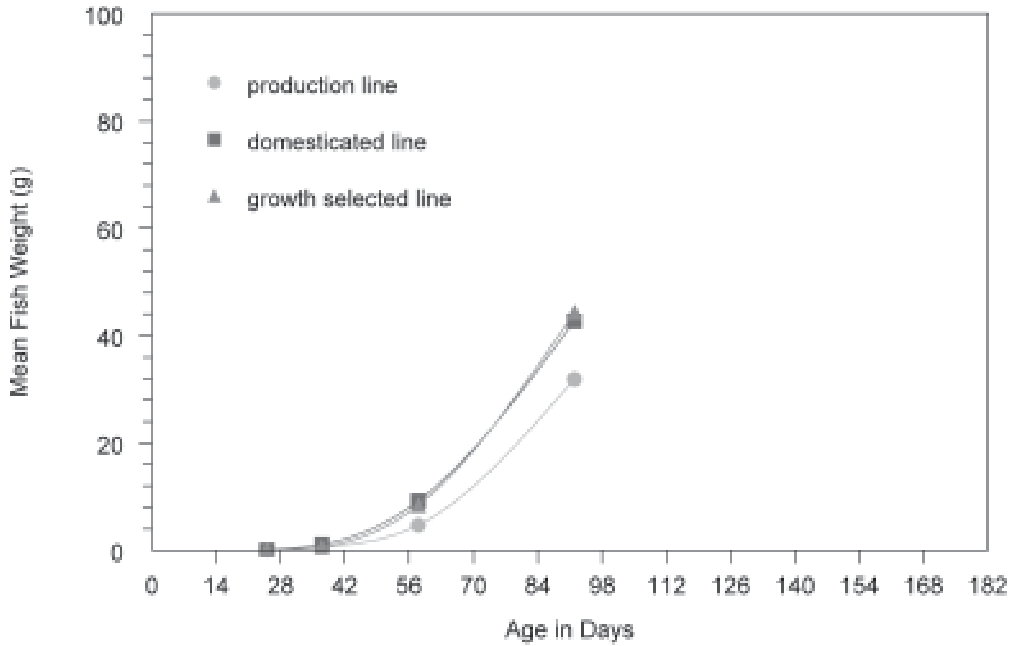
FIGURE 2. *Photographs of the fingerling tagging process. The top left panel shows technical staff at OI in the process of tagging over 2,000 Pacific threadfin fingerlings for the CTSA-funded selective breeding project. Technical staff began the process by lightly anaesthetizing fingerlings and implanting individual coded wire tags (top right panel). The fish were then implanted with a highly visible fluorescent elastomer in the adipose tissue behind the eye, marking the treatment groups: green for production controls, red for domesticated controls, and yellow for growth-selected (see lower left panel). The bottom right panel shows fingerlings swimming in the recovery tanks immediately following implantation.*

surpassed (not significant) the domesticated control group. The next three months will determine if selection for growth is translated into significantly improved growth performance to market size.

Objective 5: Gain estimate of heritability for growth and indirect effects on survival, dressing percentage, and reproduction in Pacific threadfin.

This portion of the project is scheduled post-grow-out and will be initiated subsequent to completion of the grow-out phase of control and select lines in approximately three months.

Objective 6: Preliminary evaluation of water reuse systems for maintaining Pacific threadfin broodstock.



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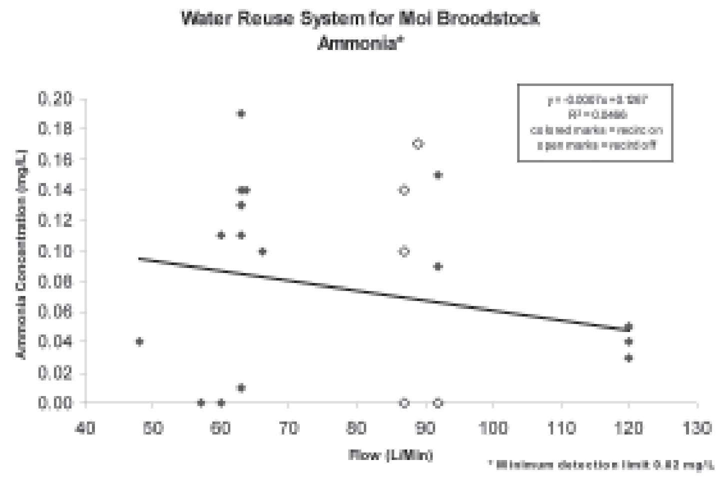

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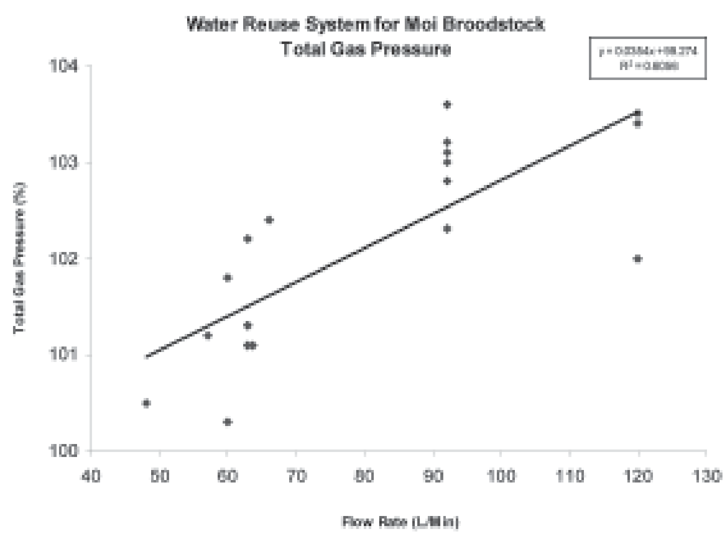

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