"*An important scientific innovation rarely makes its way by gradually winning over and converting its opponents: it rarely happens that Saul becomes Paul. What does happen is that its opponents gradually die out, and that the growing generation is familiarised with the ideas from the beginning*."

-- Max Planck

As a scientist and inventor, the above quote is my favorite among quotes by scientists. In my 35+ years of academic and scientific experience, Planck's observation has been proven over and over again.

Here we go again…

We would like to thank the Council for the effort expended in generating this solicitation. It was "yeomen's work", no doubt. We believe that all of the interested parties would agree that the Council, the ISRP, the project sponsors and the proposed project's partners were under considerable time pressure. Time "crunches" often lead to oversight and cutting corners. We acknowledge that we may have "cut some corners" of our own in an effort to submit a timely proposal that met all the Council's requirements. It appears that some of the other interested parties may have felt the need to "cut a few corners" as well. While we will *not* argue that we should have had both our proposals (The Natural Tag –TNT and Automated Scale Image Analysis- ASIA) ranked in the highest categories, "unranked" – without merit for consideration - seems "odd". If this were our opinion alone, it would be easy to dismiss this view as a matter of sour grapes on our part. However, several objective, uninterested parties have read our proposals and the reviews, and agree that the reviews (of these two proposals) were, at best, "odd". Below we address the issues the ISRP raised regarding Biopar's proposals, and offer alternate interpretations and explanations. We would ask the Council to attach our response to any public publication of our proposals. In the text below, the ISRP's comments are italicized, our response follows immediately.

The Natural Tag:

1) The proposed biometric technology is described by the sponsors as new, while at the same time described as "developed in 1985".

I wonder how Thomas Edison would have felt about such a criticism after applying for the patent for the "light bulb" 30 years after the first bulb filament experiments were conducted. TNT is a paradigm-changing technology. The facts are, as stated in the proposal, that while the fundamental concept was "developed" in 1985, the basic computing power required to perform the computationally intensive processes involved has not been available until very recently. Up until the past few years, to have the kind of computing horsepower needed to run TNT meant you had to have a supercomputer or you had to be working at a Fortune 500 company, NASA or the Department of Defense. Great strides have been made in the last few years to both speed up over-the-counter computing horsepower and processing speeds while also seeing the costs come down. Directly from the proposal: **In the last few years computing speeds and capabilities have increased dramatically while prices for these capabilities have fallen. Combine this with advances in consumer-grade and commercial digital camera technology and the market is finally to a point where it is ready for TNT to be applied on a wide scale. TNT harnesses the power of over-the-counter technology and combines it with specialized expertise.** This statement alone clarifies the Panel's implication of contradiction.

In terms of the issue of timing, Jean Baptiste Joseph Fourier, a French mathematician credited with "discovery" of the Fourier series, died in 1830, ridiculed by his peers for the "failings" (in a purely mathematical sense) of his work on the Fourier series. It wasn't until *real computing* came of age, **130 years later,** that the full power of Fourier's contributions were realized. Hos work is now the foundation for almost ALL telecommunications and digital signal and image processing. Now, we aren't comparing ourselves to Thomas Edison, Joseph Fourier, or anyone else other than to note that sometimes innovative ideas , even great ones, aren't fully realized in their time because "technology" doesn't catch up with "idea". We don't think the world should (or will) have to wait 130 years to see the value of TNT.

The "establishment-type" review given these two proposals illustrates how difficult it is to introduce a genuinely new technology into a well-established system, even when the proposal being reviewed is in response to a solicitation specifically calling for "New and Innovative Technologies". The "disapproving" tone of the Panel's comments *is clear* even to a casual reader. (We are not relying on our own subjectivity in the matter.) *What* they are "disapproving" however, is *not* clear. In the above comment, is the Panel implying that we are trying to fool someone into funding something that really isn't "new technology", or is their criticism that we haven't yet established ourselves sufficiently to request funding from a program directed at introducing "New and Innovative Technologies"? The implication is that we are either deceivers or interlopers. We, and others, find this criticism in a review of the scientific merits of a "new and innovative technology", "odd".

2) Apparently, the innovation is that the technology has not been used in the Columbia River Basin.

The not-so-subtle sarcasm of this comment can be found here-and-there throughout both of these reviews. This is not just our, the authors, observation. TNT, in and of itself, is an innovation and to characterize it in the manner chosen above seems inappropriate. Talk to any hatchery manager and they will tell you TNT is an innovation. Talk to the field biologists responsible for tracking and managing hard to tag species (they don't even have to be endangered) and they will tell you TNT is innovative. The issue of innovativeness is not one of locale or species, but whether technology can be paradigm changing and affordable. TNT is both, as every manager we have shown it to agrees. "Apparently" the Panel is suggesting that TNT is not "innovative".

3) *However, a demonstration project is already underway (pictures of fish to be transferred from an ODFW hatchery to NOAA Manchester) for the ongoing Captive Brood Stock program for Catherine Creek, Lostine, and Grande Ronde Rivers.*

This is not an accurate reflection of what was conveyed in the proposal. We understand how a cursory reading of a single sentence in the proposal *might* be misinterpreted to mean that a **TNT** project was "underway". From the proposal: "**Nez Perce-Oregon Department of Fish and Wildlife– Confederated Tribes of the Umatilla Indian Reservation Chinook Captive Brood Stock program(CBS) sub-project** is in fact <u>already underway</u>." (Emphasis applied here only.) However, a *complete* reading of the paragraph renders that interpretation completely unsubstantiated. Again from the proposal: "The project <u>we are proposing here</u> will follow these fish as they mature to spawning, demonstrating the ability of TNT to deal with how a pattern changes with growth."

ODFW and the Nez Perce and Confederated Umatilla Tribes *have an ongoing captive brood stock program* at the Bonneville Hatchery and Manchester Research Station separate and apart from what we are proposing. *That* is the project that is "underway". We assumed (apparently incorrectly), that those familiar with Columbia River Basin issues would have been aware of the Captive Brood Stock project that has been in progress for several years, and is in fact in "sunset" mode. Because the life cycle of salmon does not time itself with proposal solicitation deadlines or funding opportunities, the ODFW, Nez Perce, and Confederated Tribes of the Umatilla Indian Reservation simply allowed us to take an initial set of digital photographs, so that the groundwork would not be missed in a project that is soon to end. If rates of growth, gender differences, weight and length relationships and individual identification from their already captive stocks were to be determined, the initial images *had* to have been taken *long* before the funding for this proposal would have been provided. Biopar agreed to do (*completely* at their own expense) all of the preliminary work, including at least two more image acquisition events prior to funding availability. Seems that old adage "No good deed goes unpunished" is still around.

We do not think the full text would lead anyone to believe that this **TNT** is project is "ongoing". Frankly, given the very negative review the ISRP gave TNT it is unlikely that Biopar will expend any more time or resources necessary to travel to these facilities this year and take those additional images. Of more significance however is the fact that we, and other readers of the reviews, question why the ISRP would want to characterize our submission this way. Even the casual reader would think the TNT proposal should be disqualified for already being in use in a project *when in fact it is not*. Deception on the part of Biopar is again implied.

4) The technical background is vague with no citations to published scientific or technical literature. Given that the technology was developed in 1985, the lack of citations to publications is troubling.

The digital image analysis and database management background for TNT is very challenging, to say the least. The concepts it relies upon are beyond the grasp of all but a few highly trained people in the world. To attempt to go into any kind of sufficient detail would be well beyond a full year course at a university, much less a 15-page proposal. We would *love* to have some citations, *beyond the NSF work we have done*, but "new" means, well…NEW. It does not mean

"established". In addition, TNT contains proprietary algorithms and methods we use behind the scenes to collect and generate our matches and other data sets. None of which would assist a reviewer in evaluating the scientific merits *of the work proposed*. We cannot and will not discuss any behind-the-scenes methods or algorithms we use, just as *any* technology-based business won't open up its source code.
The scientists developing the processes used in genetic stock ID are patenting *the genome* for goodness sake.

The fact that something doesn't have citations may, and in this case does, simply mean it is new- which is what we thought the "New and Innovative" program was all about. Forty years of literature exists on image analysis and pattern recognition, biological growth patterns and the like. Perhaps we should have included references to such topics, but they would have been of little assistance in evaluating the merits of the work proposed.

5) A National Science Foundation grant is cited by number, but background information on the results of this grant is insufficient.

We're not even sure what this statement/criticism means. All we can assume is that the reviewers didn't consider NSF's evaluation of our fundamental research "sufficient". We could have included the report in total, as we did in our ASIA proposal, but as the Panel made patently clear in that review, they didn't even consider it because it was an "attachment". This is precisely a case of "we're damned if we do, and we're damned if we don't". Again we would point out the page limits and that we felt it more important to discuss what we were "going to do" (the methods and objectives of each sub project), instead of what we "had done". Please keep in mind that we had to describe the field work for six separate sub-projects. We are attaching a copy of the NSF report, should the Council care to read it.

6) The proposal addresses a general need to accurately identify individual organisms with non-invasive technology but does not present a convincing case as to how the work will further the goals of the 2000 Fish and Wildlife Program, subbasin plans, or other plans. No specific problem is identified.

Seriously? Aren't the largest debates surrounding the Columbia System and its dams one of "people versus fish", "the impacts of the dams on the fish", "what's happening to the fish?" Doesn't every sub basin plan, and the mainstem plan for that matter, address in great and painstaking detail all of the problems facing salmon, frogs, birds, etc? Don't all projects that are undertaken with BPA dollars for the benefit of species assistance or mitigation have to provide a measurements and evaluation component for how they will determine success or failure for a project? How does one collect that data on fish or animals without performing some sort of population estimate, mark recapture event, etc? Would any other tagging technology such as PIT tags or coded wire tags be required to explain exactly how their tagging *technology* would "further the goals of the 2000 Fish and Wildlife Program?"

We ask rhetorically; "How much is being spent annually to clip fins, insert coded wire or PIT tags, mark fish with otolith tags or perform some other identification on them for upstream, downstream and returning spawner evaluations?" The Nez Perce Tribe and US Fish and Wildlife themselves have a court ordered release of approximately 200,000 hatchery raised fish every year that must be let go *with no form of tag at all*, including no adipose fin clipping. How else are resource managers supposed to track these fish unless they use "new and innovative" digital imagery for individual ID that does not mark or otherwise physically change the fish in any way?

There is not just a *"general need"* for better tagging and data collection mechanisms in the system – there is a screaming need, in painstaking, laborious, arduous detail, spelled out over, and over, *and over* in several *thousands* of pages of mainstem and sub basin plans adopted by the Council. In fact, the sub basin and mainstem plans have very specific goals of identifying new tagging methods for fish and animals. Perhaps the ISRP was correct that we did not spell out in great detail (in 15 pages) how the technology will work, but we would hope the Council would agree that there is most certainly a push for better data collection and tagging alternatives – especially one that is faster, cheaper and less invasive than current methods.

Not a convincing case for better tagging technology? "Odd".

7) The sponsors would like to collaborate with numerous agencies in the Columbia River Basin and have established relationships with groups ranging from high school biology classes to government hatcheries. Details of imaging technology and data analysis and evaluation methods are insufficient to judge the scientific or technological merit of the six proposed demonstration projects.

We would "like" to???

Is the Panel suggesting that we have duped 20-some-odd biologists and eight different government and tribal agencies, many of whom have decades of scientific and field experience in the very subject fields we are discussing?

Is the Panel suggesting that its time-constrained review of the proposal provides a greater insight into the merits of TNT than the year-plus these other biological professionals spent actually evaluating the merits of the proposed work?

The Panel's choice to use the word "like" is hardly subtle in suggesting that we didn't even talk to these State and Tribal partners before putting this submission together; that we somehow secretly "hoped" they would open their doors to us if we showed up with "free" BPA money.

Suffice it to say, the design of the TNT proposal – put together with input from all of our project sponsors - was intended to demonstrate that TNT can easily be deployed, across geographic zones, across different taxonomic groups and by groups with varying degrees of education and training. To put it another way given the ISRP's comment about having a lawyer involved: "If a high school student can do it, then it's likely that even a lawyer can do it."

Taking photos at select intervals of time and comparing them to previous photos for "matches", plus checking those proposed matches against the unique PIT tag number inserted into each test subject is not a complex concept. One doesn't need to understand (or have rehashed) the technical elements of pattern analysis and machine intelligence. Neither does the proposal lack specificity sufficient to demonstrate that TNT works across the various animal taxa being examined. It is unclear what the Panel needed in detail beyond what we provided in the submission and beyond what basic scientific method principles one would expect to find in any field work. What one might reasonably assume from the above comments (in total so far), is that the Panel was looking for "the status quo" in proposals. We acknowledge that while we did not provide that exactly, we most certainly provided fully sufficient information *to evaluate the merits of the work proposed.*

As for the technical issues surrounding image analysis and the data base, we put it in the proposal thus: Scientists have been using pictures of whale flukes for years to ID and track individual whales. The same principles have been applied to other animals, such as frogs, zebras, the big cats. Managing databases is nothing new. What is new is applying all the techniques under one very fast and *completely automated system* so that it is useful and available to *all* resource managers, system-wide.

8) The technology might be useful as a method for scientific research proposals that address a specific issue, where there is a need to identify individual organisms without invasive procedures.

A "nice", but "left-handed" compliment. Without going into greater detail than what was said above, TNT has applicability across virtually all animal species in the system, not just an animal that has a specific issue. But if one wants to focus on animals with specific issues, then perhaps salmon would be specific enough. We believe that being able to tag more salmon for less money is incentive enough for the Council to want to field-test a new technology purporting to do so. Doing it without tag loss should "pile on" interest for a test. Automatically identifying the gender of out-migrating smolt, rates of growth and specific weights and length at any life-stage furthers justification. Collecting the data without having the kill the animal or install a $150,000 machine at every sampling station "piles on" more "use". One would think the "specific issue" of salmon alone should cause the Council to be interested in field-testing TNT. Especially in light of the modest (relative to the "highly ranked" proposals) level of funding requested. No proposal submitted in response to this solicitation comes close to offering the "bang-for-buck" that TNT and ASIA do.

9) The sponsors do not present a convincing case that their proposed demonstration projects, *objectives, methods, and products will provide any direct improvements in the survival or productivity of Columbia River fish or wildlife species.*

Perhaps the Panel misunderstood our proposal. Biopar is not in the business of producing fish, repairing habitat, making management decisions about the resource, or performing other mitigation to help species recovery. Rather TNT is designed specifically to *help* in all these areas at the fundamental level of data collection. In contrast to the misdirection of the review comments, answers to the following basic questions may help put the proposal in *proper* perspective: 1) What are the specific concerns currently in the Columbia System; and 2) What methods are currently used to determine animal abundance and perform population dynamics statistics. In almost every instance TNT can be used for projects designed to answer those questions, and do it faster and cheaper. Fundamentally, it is the tagging technology of the 21st century. But that's only the "tagging" element of TNT. The integration of the data allowing mining of a 'cornucopia' of information ranging from the tiniest drainage to an entire eco-system, is staggering.

At least as important is the following: Existing tag manufacturers are not asked to "improves survivability"; they are asked if their tagging method allows the managers to collect the data needed to determine survivability. Why then is TNT being held to a different standard? This *technology* is not about improving survivability or productivity, it's about improving a method which results in improved insight, which results in improved action. No tag – be it physical or digital – ever saved a species. Biologists, with good data, make the critical, species-saving decisions

. We acknowledge however, that we do not get to determine what makes a "convincing case" to the ISRP. The ISRP has stated unambiguously that they do not consider "a convincing case made" for TNT. That's "odd".

10) Although the sponsors claim successful application of their technology to fish (especially grayling), there are no peerreviewed citations in their CVs.

This is similar to the criticism the ISRP's made in its review of our ASIA proposal so we will summarize our response again here. (And actually, *per the proposal*, the fish most studied using TNT is *O. mykiss*.) We didn't realize that the weight of the CV would be a determining criterion. Levity aside, this solicitation was intended to seek out new and innovative technology – the operative word being *"new"*. As such, there aren't any publications regarding this specific subject because it's *new*. That's the nature of "new", innovative, paradigm-changing technologies. CVs with long lists of publications unrelated to the matter at hand are not Biopar's "style". This, "burden", we acknowledge is self-inflicted. It, however, is a *philosophical* issue, not one that we feel should be a matter of consideration when reviewing the scientific merits of a proposal for "New and Innovative Technology Projects". We remain of the opinion that the proposal's scientific design stands on its own merits. Perhaps the Panel would have preferred that we had included lists of publications dealing with image analysis, pattern recognition, algorithm development, and database management techniques or other similar scholarly works – but we doubt seriously if they would have actually had the time to pull the papers, read the submission and familiarized themselves with the detailed, specific, and highly mathematical underpinnings of these issues. Partly because that is not really their job, and partly because, based on their own comments, they wouldn't have had time to do so for all of the submissions received. We understand that nobody actually reads these citations, they are only a *convenient* (albeit superficial), method to qualify an author. We submit that the CVs provided (if actually read) fully qualify the authors, and that the proposal's scientific design should be decided on its on merits.

11) If the results of the demonstration projects were published in a peer-reviewed journal, the findings would have some long-term benefits to numerous focal species.

Again, this is similar to the critique we received in our ASIA submission, and so our answer will be the same. However, since the Panel has inserted the term "long-term", we feel it necessary to address that first. Every "long-term" project ever conducted in the history of mankind had to start "in the short term" and without any "history" behind it. This criticism might be apropos if the proposal wasn't in response to a solicitation for "New and Innovative Technology".

This is once again, in our opinion, a semantic issue. It is our opinion, that the request in the solicitation regarding "Information Transfer" was most appropriately addressed by those elements *within our control to accomplish*. Publication of the results of this work in a peer-reviewed journal, while highly desirable, is *completely* outside our, or any other project sponsor's, ability to claim as a certainty. We felt, and continue to feel, that putting down what we **hope** *might* happen, is at best, wishful thinking, and has no place in a rigorous proposal. (It's clear the Panel disagrees on this.) No other project sponsor can *guarantee* their results will get published. We would be very pleased to have our work so recognized. Nowhere in the proposal did we shut the door to that or exclude that in any way. On the other hand, what we *can* be *certain* of is lecturing on the results, making the data available for regional public data bases, and working with our project sponsors (states and Tribes) to assist them in disseminating the information as well. We find it a bit "odd" that the ISRP should imply that we wouldn't want our results published in peer-reviewed journals. We would ask the Council to consider if ours is not an appropriate response to the inquiry about "Information Transfer", and that the ISRP's above criticism isn't "odd".

12) They might be especially relevant to rare and endangered species where there are actually only a few hundred living animals.

Actually this is precisely backwards. If you only have a few hundreds living animals, or animals that are very rare, then you can get away with taking pictures and having humans make matches from a collection of photographs. This is what has been happening for 30 years with whales and frogs.

TNT is most valuable when the library of images is so large humans could never make the match (trying to match a photo of a fish from a library of 1 million such fish released from a hatchery) or for animals where no effective, (or only poor, behavior-modifying, invasive), tagging methods exists, such as for certain insects, snakes, frogs, crabs, etc. The fast, automated process of TNT allows such “problem” creatures to be “tagged” like never before. Certainly TNT can still be helpful for rare species that you do not want to mutilate in tagging, but it is in no way limited to such applications, and there is *nothing* in the proposal to suggest that such limitations are the case. Likewise, being able to automatically derive other data, such as gender, growth, weight and length and other useful information from photographs, has proven to be of great interest to resource managers, including the state and tribal partners in our submission, who have seen the technology in action.

13) The inclusion of a lawyer as a co-principal investigator on this project is not adequately justified.

Here the ISRP stepped *way* over the line. The comment is not only “odd”, but highly inappropriate. Most significantly, combined with the above “odd” comments, it strongly suggests that the reviewer(s) were less than objective in their consideration of this proposal. First, the ISRP does not get to choose whom Biopar has as business partners. More importantly, if the reviewers had bothered to actually read the CVs with an objective eye, they would have seen that Mr. Stiefel is trained as a Chemical Engineer, otherwise referred to in industry as a Process Engineer. For the benefit of those unfamiliar with this technical discipline, chemical/process engineers are trained in the design and maintenance of chemical and physical processes for large-scale manufacture or processing. It requires detailed understanding of processes, creativity in application and invention, and solid math skills. We would hope that it would be clear to the most casual observer that TNT is nothing if not *process*. We did not discount Mr. Stiefel’s involvement with Biopar simply because he took the time and expended the effort to go to law school. There is **NO** *good* reason why the ISRP would feel it appropriate to comment in this manner, especially given Mr. Stiefel’s training as an engineer.

In Summary:

This technology, digital image ‘tagging’, is coming. We had hoped that this source of funding would have provided the resources needed to get these technologies in use in the Columbia River Basin. We have no shame in admitting our disappointment at these reviews. We are also disappointed for what might have happened for the high school students that would have participated; we are disappointed for those that wish to implement non-invasive tagging of the **millions** of fish that are tagged in the drainage every year; and we are disappointed for those that **have no existing method** of tagging the species they are studying. Considering what we proposed, and the modest amount of money we were requesting, (most of which was going to our collaborators, not us), we were hopeful that reviews for a solicitation for “New and Innovative Technology” would have made the effort to evaluate the actual merits of a proposal instead of simply checking to see if it fit the status quo for traditional proposals. The truth of the matter is: The risk that both TNT and ASIA represent relative to the extraordinary benefits they proffer is miniscule. Programs that genuinely consider themselves as supporting of "new and innovative technologies" should expect to have to take on some modicum of risk. The TNT and ASIA proposals suggest no more than a modicum of risk. Nothing new in any of life's endeavor's happens without letting go of the "established". Isn't "new and innovative" the antithesis of "established"?

Again, we ask the Council to contact our project sponsors listed in our proposal to discuss their desire to see this cost effective and innovative technology deployed (and their confidential opinions of the appropriateness of the reviews). They are professionals who deal with the "on ground" realities of natural resource data collection, every day. They would not have agreed to let their names be added to our proposal, or to work with us on this project, if they felt this project lacked merit or that our "lawyer" was getting in the way.

We grant and acknowledge that the ISRP team had many projects to sift through with little time for the task. Nonetheless, other disinterested third-parties agree that Biopar reviews were replete with "odd" criticisms and in one instance (in both proposals), highly inappropriate comments. We are not asking for a "new" review by the Council. We would simply ask the Council to read the proposals, **not** to scientifically review them, but rather to ascertain for themselves if the reviews provided by the ISRP of the two Biopar proposals, provided the Council with the assistance they ask for in determining the best proposals to recommend to BPA for funding for "New and Innovative Technology".

In summary, it is our opinion, and the opinion of disinterested third parties that the tone of both the TNT and ASIA reviews were condescending (in some cases sarcastic), with vague criticisms, most of which, (we acknowledge not all), have little relevance to the scientific or technical merits of the proposed work. It is clear to us that, considering the ISRP's reviews and the program's available funding, that funding through the New and Innovative Technologies solicitation will not be provided for these two proposals. We do hope however, that we have generated sufficient interest within the Council; that the Council might consider discussing with us or any of our regional partners, how all of us interested in getting the extraordinary capabilities of both TNT and ASIA in use as quickly as possible within the Columbia Basin, might accomplish it.

Thank you for your time and efforts.

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Preliminary Report:

The basic research goal of the Phase I project was to demonstrate that image processing and pattern recognition techniques could be applied to images of fish for the purpose of extracting and quantifying a pattern unique to the individual, that could be used to identify the individual throughout its lifetime.

To that end, five technical objectives were presented in the Phase I proposal. They were:

1) Create a unique mathematical description of the pattern of spots on a fish;
2) Determine the selection criteria for the spots;
3) Develop accept/reject criteria used to identify the unknown as either a new fish, or a recapture, including methods to identify spot “drop-out” and “generation”;
4) Determine within-fish variability of the feature vector; and
5) Construct a field applicable, physical prototype.

Additional secondary goals were: When do young *Oncorhynchus* species start depicting their permanent spots, and how much did growth affect the ability of a machine to correctly extract the pattern of those spots automatically?

Arrangements were made with the Alaska Department of Fish and Game (ADF&G) to use their hatchery at Ft. Richardson, in Anchorage Alaska. The hatchery had 800 juvenile, ‘double-x chromosome’ male *Oncorhynchus mykiss* (Rainbow trout) available as subject animals. The 800 fish were kept in four tanks designated E1, E2, E3, and E4. Respectively, the tanks held 200, 199, 196, and 195 fish. At the outset of image acquisition, the fish were approximately 210 days old. Average length was 155mm, and average weight was 35.7g. Image acquisition commenced on 02/03/03, and ended on 04/01/03. A final sampling event was conducted on 07/02/03 after the fish in all four tanks were aggregated and moved to a single outdoor raceway.

The fish in tanks E1 and E2 were designated as controls for evaluating lethal (mortality) and sub-lethal (growth rate) effects of handling and photography. The fish in tanks E3 and E4 were photographed.

Figure 1.

The following explanation is provided to illustrate the relative importance of these fish to the hatchery, and how the image acquisition schedule was influenced.

These fish were part of a pilot study by the ADF&G to evaluate the process required to produce sterile females for release into local lakes and streams for recreational fishing. Because of public concern about genetic contamination of wild stocks from hatchery-planted fish, the ADF&G was examining the feasibility of producing sterile fish for stocking in recreational fisheries. Double-x males were created by thermal shock of the eggs shortly after fertilization. Once sexually mature and of appropriate size, the fish will be sacrificed and the testes macerated to harvest the sperm. (As males, they have no oviduct.) When used to fertilize eggs (which are of course x-only) this x-only sperm will produce all females. Thermally shocking *these* (f2) fertilized eggs will produce ‘triple-x’ females, which are sterile, but manifest the same or slightly better growth rates as ‘normal’ females. The entire process will take more than three years to complete. Because of the substantial investment in time and resources, the hatchery did not want them subjected to any more stress than was absolutely necessary. Acquiring images would require anesthetizing the fish, and when anesthetized, fish often quit feeding for a day or two. If they don't eat as much, they don't grow as much, and they won't reach the desired size by the desired date.

It was decided that experimental design needs could be met by sampling each tank every other week. In other words, on Monday of every week all the fish from *either* E3 or E4 were photographed. The following Monday the fish from the other tank were photographed. In this way, a sufficient number of images could be acquired, and each individual fish was subjected to less handling. Six thousand fifty-four *initial* images were acquired from 391 fish.

In preparation for photographing, approximately five fish would be removed from the brood-tank and placed in one of two anesthetic baths. The anesthesia, tincture of clove oil, was provided by the hatchery. In approximately 3 minutes, the fish would be suitably anesthetized so that pictures could be taken without the fish struggling. When the fish in the first bath were belly up, five more fish would be added to the other anesthetic bath. At least three pictures of each fish, one full-fish image of each side, and one image of the top of the fish's head were taken. Occasionally additional pictures were taken. After having its pictures taken, a fish would be placed in a recovery bath with fresh water and artificial aeration. After recovery (approximately 10 minutes), the fish would be segregated in an isolation bag suspended in the brood-tank. Once all the fish were removed from the brood tank and photographed, those segregated were returned to the brood tank.

There was no opportunity to alter the lighting regime in the brood facility, therefore the overhead fluorescent lights provided the only source of light. Initial pictures from the first two sampling dates were taken with the fish simply placed on the platen of the camera stand (02/03/03) or in a cradle (02/10/03). However, the results were fundamentally useless for *automated* spot extraction because the fish were so small that the specular reflections from the over-head lighting obscured too many spots. The solution was to keep the fish submerged so that the water would diffuse the light and prevent specular reflections from the fish. (Specular reflections from the water's surface were eliminated from the image frame by positioning the camera and fish-holding tank as needed.) Specular reflection while present, was not an issue for the lateral images, so the fish was not submerged.

The Sony F717 camera using 128MB Memory Sticks was capable of storing approximately 65 1960 pixels x 2520 pixels x 24 bit color images. Using jpeg compression, the stored size was approximately 2MB per image. Once full, the Memory Stick was downloaded to the hard drive of a laptop computer on site. Later, the images were written to CDROM for long-term storage, and to a DataFlow server for short-term retrieval and analysis.

Figure 2.

In figure 2, the anesthetizing baths are in the foreground; the camera stand, platen and Plexiglas fish holding tanks are in front of the blue tub used for recovery.

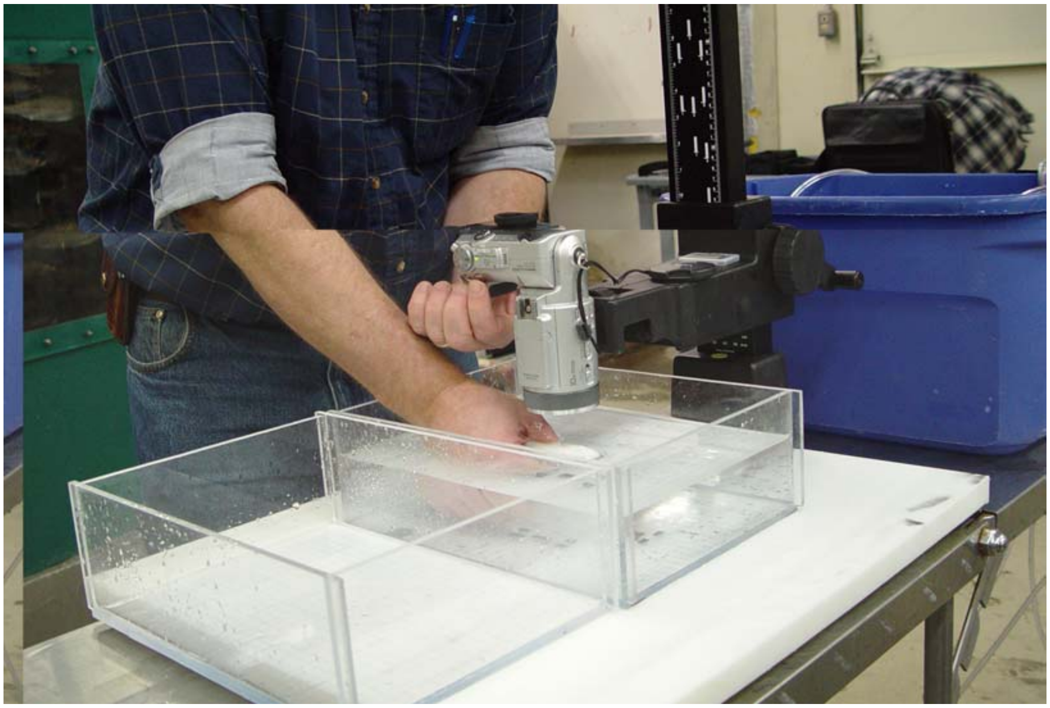

Figure 3.

In figure 3, you can see a fish being held beneath the water, and the range at which the dorsal head pictures were taken – approximately 7cm.

The camera, seen mounted on the black stand, can be positioned and indexed anywhere within a 1m range on the stand. The two Plexiglas tanks held the fish during photography.

In addition to the double-x males held in these four tanks, there were also arctic charr (*Salvelinus malmo*) and ‘normal’ *O. mykiss* being brooded in the same room. The charr were held in a 6’ diameter tank, and the rainbow trout in a 12’ diameter tank. Each tank had a large viewing window through which pictures could be taken. Several hundred images were taken with the intended purpose of simulating the conditions one might encounter at a dam-passage facility.

Figure 4. *O. mykiss*

Figure 5. *S. malma*

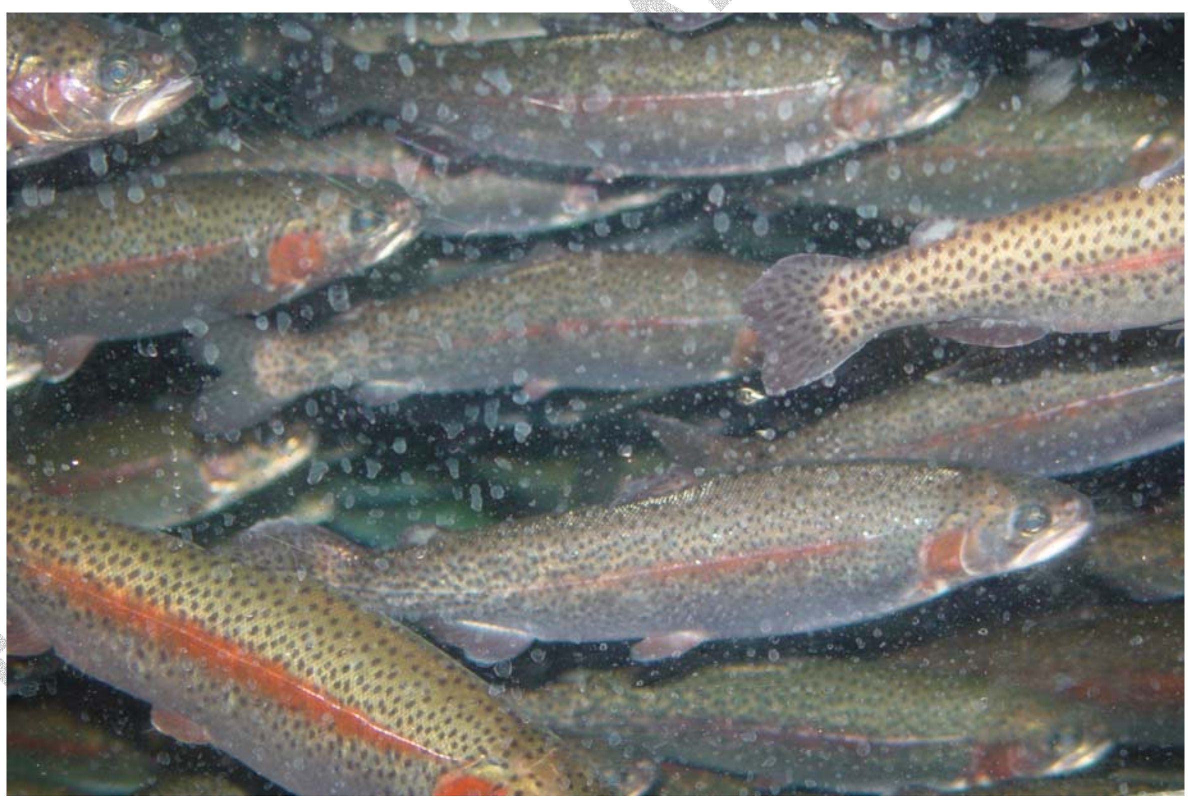
Figure 6. *O. mykiss*

Figure 7. *S. malma*

Figures 6 & 7 are representative of the image quality possible with available light and the Sony F717. As poor as these images are, the salient features of the fish are clearly obvious. As such, they are easily extracted. Training a machine to extract them without human supervision or preprocessing is non-trivial, but is also not overwhelming.

The first picture taken was the dorsum of the head, followed by the full lateral view of each side. The image of the head was taken at a focal range of approximately 7cm; the focal range for the lateral images was 30cm until the last sampling date (04/01/03) in which it was 40cm. This provided a simple uniformity that facilitated post-acquisition processing.

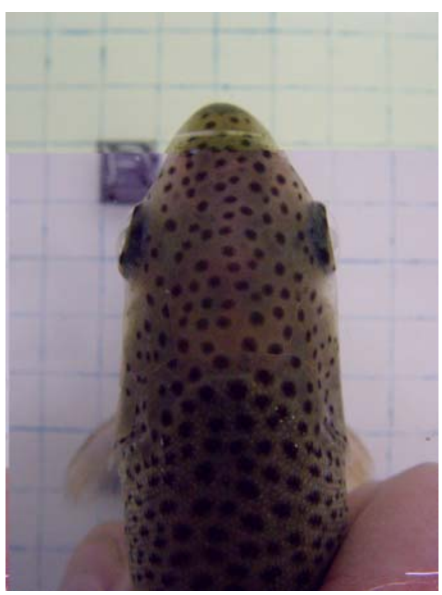

Figure 8. Head dorsum

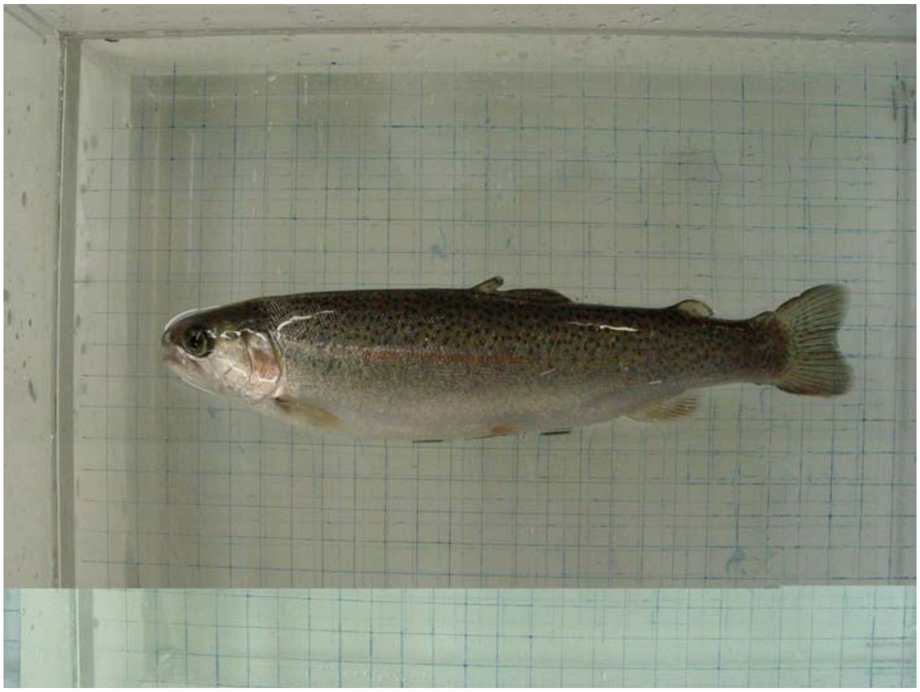

Figure 9. Left side lateral.

The total process of taking approximately 600 pictures, including setup and take down, took about 6 hours - about 100 images, per hour. This is considerably more time than would be required in a typical field deployment where more fish could be anesthetized at once and fish could be released directly back to the source body of water without concern for mixing with unphotographed fish.

Once the images were acquired and properly archived, three processes were started. In order to determine the effect of growth on the movement of the spots, the growth had to be measured. As can be seen in the above figures, there was a 1cm grid etched on the bottoms of the fish holding tanks. Using the number of pixels per cm in each image, the length of the fish was easily taken directly from the image. Second, all the head pictures from a given day's sampling had to be matched 'by eye' with all the head pictures from all previous sampling events for the specific tank – either E3 or E4. This matching provides the ground truth against which the automated matching was compared. The third task extracted other morphometric information from the images. That information was the distance and angle between 15 pre-selected points on the head and lateral views. On the head image the two values were intra-nares distance, and distance from a line drawn between the nares to the terminus of the skull. On the lateral view, the 13 points were; 1) the nares, 2) the focus of the pupil, 3) the center of he pupil, 4) the intersection of the lateral line with the posterior of the cliethra, 5) the insertion of the pectoral fin, 6) the insertion of the dorsal fin, 7) the insertion of the pelvic fin, 8) the anterior-most point of the adipose fin, 9) the anus, 10) the dorsal point of the caudal peduncle, 11) the terminus of he lateral line at the tail, 12) the ventral point of the caudal peduncle, and 13) the fork of the tail. Distance and angle measurements of the adjacent points allowed the construction of a box-truss diagram of the lateral view of the fish. Analysis of the box trusses provided detailed information of the growth of the fish. It was thought by some reviewers that detailed growth information would be necessary to compensate for spot movement with growth. That was not the case. An extraordinary amount of time and effort was spent on acquiring the data for this analysis. Non-automated extraction of length data for each sampling event (200 images) took approximately 6 man-hours. Approximately 40 man-hours per sampling event (200 images) were required to hand-match the head images. Some 600 man-hours were spent on acquiring the data for growth analysis.

After the 03/03/03 sampling event, it was possible to start testing the spot extraction and pattern matching algorithms. A sequence of image processing methods (filters, etc.) were developed that would 1) obtain information about the spots, and 2) create the masks to permit automated spot location. A gaussian convolution kernel was applied to reduce high frequency noise, followed by a contrast diminishing 'flattening' filter. The image was then thresholded rendering a binary mask that could be applied to the image and the spots extracted. Figures 10 and 11 are derived from the fish in Figure 8.

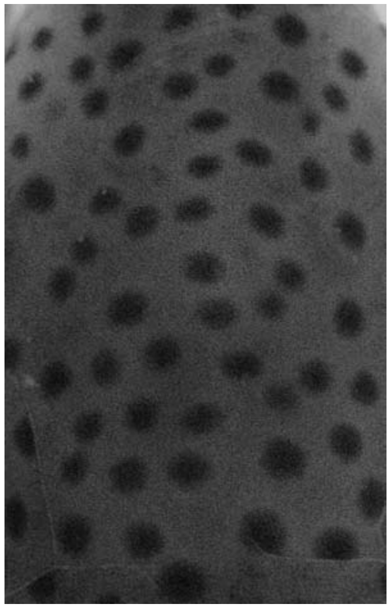

Figure 10.

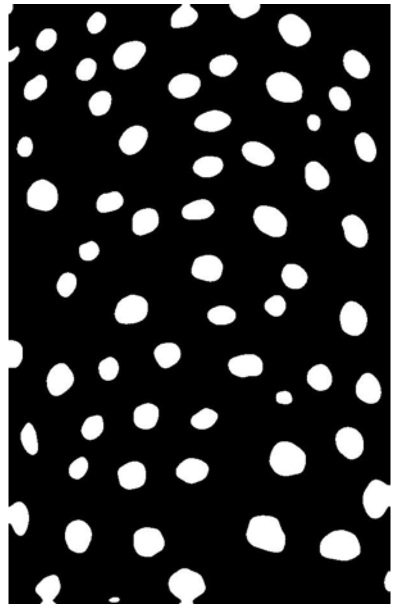

Figure 11.

Exhaustive evaluations of matching algorithms that relied on extracting the position of the spots and creating feature vectors from that spot position information, demonstrated that feature vectors so constructed were too sensitive to rotation of the fish on its longitudinal and vertical axes, spot selection, spot drop out and spot generation. As a result, the best fully automated matching accuracy that could be achieved was approximately 50%. The method ultimately used was sufficiently robust, that it eliminated any requirement to "compensate" for spot dropout or generation. This is not to suggest that artificial dropout or generation as a function of the spot selection algorithm was eliminated by this method. Nonetheless, the need to develop a technique to overtly address spot dropout or generation was eliminated. Considering that processing time is a significant limiting factor, eliminating the added processing required to address spot dropout and generation represented a significant improvement in processing time. Furthermore, the new method was the most effective method (highest accuracy) employed – 90% unsupervised, and 100% supervised.

On 06/01/03 the fish were moved from the brood-tanks in the brooding facility to outside raceways. The fish from all four tanks were combined in a raceway. A final set of images was acquired on 07/02/03 from the raceway containing the fish from all four indoor tanks. The images were acquired in conjunction with a scheduled hatchery sampling event. The image acquisition system (IAS) was set up in the raceway, and images were taken as the hatchery staff completed their weight and length measurements. Environmental conditions could hardly have been worse. It was raining, the IAS was setup in the raceway and therefore the IAS was in the water, the lighting could not have been worse and because of the nature of the other activities being conducted, there were myriad bubbles trapped in the surface of the water and therefore recorded in the images. The image processing and pattern matching algorithms were applied to the 07/02 images, and the results were pleasantly surprising.

Regarding image quality, it was stated in the Phase I proposal that, "A flashlight in the middle of the night in a pouring rain would be completely sufficient." The Phase I results have not caused us to retreat from that position. However, clearly "speed is of the essence". The higher the quality of the images, the less time is required to process the images. The image below illustrates how bad an image can be and still be capable of being correctly matched. While the image was not taken in the middle of the night with a flashlight, the PI was standing waist deep in water and in the rain as stated. Had a flashlight been available, the image would have been better.

Figure 12 (note surface bubbles)

Figure 12 illustrates the poor quality an image could be, and still match using the newly developed method.

We were also able to field-deploy the IAS at an operating ADF&G coded-wire tag project. There, we acquired some 3000+ images of coded-wire-tagged out-migrating coho salmon (*O. kisutch*) smolt. These fish will first be returning to spawn in the fall of 2004, but majority will return in 2005. Only images of the tops of the heads were taken.

Results:

Reiterating the five technical objectives: 1) Create a unique mathematical description of the pattern of spots on a fish; 2) Determine the selection criteria for the spots; 3) Develop accept/reject criteria used to identify the unknown as either a new fish, or a recapture, including methods to identify spot "drop-out" and "generation"; 4) Determine within-fish variability of the feature vector; and 5) construct a field applicable, physical prototype.

We address each one in order.

1) Create a unique mathematical description of the pattern of spots on a fish. This goal was accomplished several times over. The initial work in this area was performed with arctic grayling (*Thymallus arcticus).* Grayling have prominent lateral spots that lend themselves well to the spot location-based feature vector. The cephalic spots of *Oncorhynchus sp.* do not. The best result derived from the spot location-based feature vector was 54% correct. The table on the next page presents the matching success for each sampling event as well as the overall matching accuracy.

2) Determine the selection criteria for the spots. Both trial and error and CART (Salford Systems), a tree-induction package familiar in both statistical and machine-learning communities, were used to determine the optimum spot selection criteria. From among a suite of 54 spot characteristics, eight (Area, Box Area, Major Axis, Minor Axis, Box XY, Minimum Density, Roundness and Number of Dendrites) were determined to be the most useful spot selection criteria. (It should be clear that from a purely statistical perspective, these criteria are not independent, but the fact is, success rates *decreased* if even one of these criteria was excluded.) All images were converted to 8-bit grayscale format to perform threshold operations. Converting to grayscale eliminated any information other than intensity that was available from the three individual color planes. The three (RGB) color planes may yield useful data with respect to spot selection. Preliminary examination of each of the RGB planes reveals that the energy in the blue plane is distributed over a narrower bandwidth, has a significantly higher maximum and is quite separate from either the red or green.

This implies that pre-filtering at the lens, specific artificial light sources, and/or post-acquisition color domain filtering is likely to improve spot selection in fully automatic mode.

	Matching Thresholded Images	Including missed images picked up on other dates	Matching version 1.0 new images		Matching version 1.1 new images	
04/01 vs 03/17	69.19% 128/185	+17 from 3/3 + 8 from 2/18 153/185 = 82.7%	78.38 145/185	+12 from 3/3 +5 from 2/18 162/185=87.57%	78.38% 145/185	+17 from 3/3 +7 from 2/18 169/185=91.35%
04/01 vs 03/03	60.54% 112/185		63.24% 117/185		70.27% 130/185	
04/01 vs 02/18	52.43% 97/185		40.54% 75/185		44.86% 83/185	
03/17 vs 03/03	63.35 121/191	+26 from 2/8 147/191 = 76.96%	70.16% 134/191	+21 from 2/18 156/191=81.68%	83.77% 160/191	+13 from 2/18 173/191=90.58%
03/17 vs 02/18	50.26 96/191		48.17% 92/191		56.02% 107/191	
03/03 vs 02/18	49.74 97/195		43.09 78/181		(processing)	
(E3)						
03/24 vs 03/10	74.23 144/194	+11 from 2/24 155/194 = 79.9%	80.63% 154/191	+6 from 2/24 160/191=83.77%	86.39% 165/191	+3 from 2/24 168/191=87.96%
03/24 vs 02/24	41.75 81/194		23.56% 45/191		34.55% 66/191	
03/10 vs 02/24	35.05 68/194		25.27 47/186		33.33% 62/186	

3) Develop accept/reject criteria used to identify the unknown as either a new fish, or a recapture, including methods to identify spot "drop-out" and "generation". Rendered moot by use of different algorithm.

4) Determine within-fish variability of the feature vector. Because a "zero-tolerance" policy was adopted with regard to false positives, the variability of the feature scalar took on a different level of importance. In other words, it had no value in assisting with threshold selection. It would only be useful to know the variability if it was very high, and as a result, the value selected as the threshold necessarily excluded correct-match energy values to prevent any possibility of a false match. In such a scenario, the variability would allow a probability-of-failure statistic to be calculated. In an absolute technical/mathematical sense, it is true that the value derived from the lowest-valued comparison could be used to establish the match threshold. However, what was found was that the cause of a low feature scalar value in a given comparison was due to poor image quality, not natural spot variability due to growth or other "natural" phenomena. In other words, the reason a correct match was not detected was not because the threshold selected excluded true matches of naturally variable fish, but rather because image quality was so poor that the machine could not autonomously identify spots. Nevertheless, the variance of the threshold values is presented. The mean correlation coefficient was 0.327 and the standard deviation was 0.088, yielding a coefficient of variation of 24.3%.

5) Construct a field applicable, physical prototype. A fully operational TNT prototype was built and tested as part of the basic Phase I research, and today DataFlow is capable of providing TNT as a commercially-available service. The TNT prototype consists of the Image Acquisition System (IAS), plus the service bureau system at DataFlow (known as the "TNT System") being hardware as well as the software that processes images and executes pattern matching/recognition algorithms.

Although the 600-plus man-hours spent collecting and analyzing the box-truss morphometric data was not necessary for compensating for growth, it provided a separate, unexpected benefit. Since we had both length and weight data as well as the 13-point box-truss data, we were able to evaluate the precision of estimated weights of the fish from the autonomously derived, morphometric data. While the point estimate of the weight derived using the classical length/weight relationship was not statistically different from the point estimate of the weight derived using a sub-set of the box-truss data, the estimates were more precise.

Design considerations for the TNT production environment (to be implemented post-Phase I):

First and foremost: processing speed. As of 6/30/03 processing time on a 2.08 GHz Dell desktop and in fully autonomous mode is approximately 395 seconds (6 minutes 35 seconds) per image. Out-migrating smolt salmon and steelhead projects employing TNT will involve no less than 50,000 images per year for at least two years before a recapture is expected. After the first recaptures, another three years will pass before all (statistically speaking for *O. kisutch*) of the originally marked fish are dead. That means that in any given year after recaptures commence, at least 100,000 images will be processed looking for matches. At 395 seconds per image, that's approximately 22 days of 24-hours per day processing. Since it is anticipated that there will be many projects of this size and larger during the first few years of implementation, it is clear that the current level of processing speed and power is insufficient to the task in any practical sense. PCs and laptops are simply not up to the task, and are not realistically even close. Improving processing speed by 50-fold reduces that 22 days to 10.5 hours. Reduced 100-fold, it's less than one work-day. In the TNT production environment, customer demand for a dramatic reduction in the time required for results reporting (as compared with existing methods) will be met.

This increase in performance will be achieved in two ways: (a) optimizing and compiling the prototype script to take advantage of multiple processors and more efficient executable code, and (b) distributing the processor load over multiple computer systems.

The current prototype is written in a scripting language designed for mathematical processing. It is convenient for prototype development, but is far from optimized. Since the scripting environment can also generate alternate source code that can be processed by an optimizing compiler, this will be the first step in improving the analysis performance. As the supporting code is developed the entire analysis system will be recreated as multi-threaded. This will allow the software to take advantage of multiple processors on a single computer system. It will also be designed to allow for the execution of more than one instance of the software on a single computing system to take advantage of possible communications or general processing latency. These changes will make analysis efficient for each computing system used for analysis.

Distributing the processing load over many computing systems scales the overall performance to the next level. DataFlow's senior technical staff has designed a system simple in structure and implementation. Dual process managers monitor and distribute work to process workstations that collect raw data from database servers. New process workstations can (and will) be added, as greater performance is required. A proper balance between process workstations, database servers, and process managers will allow the analysis system to process all the images required of it in the foreseeable future.

The estimated increase in performance is 50 to 100 times the prototype's performance. When the system is in production the server and process workstation pool will be matched to the expected load. This design allows rapid adjustment to the level of processing needed and assures that the demands required of the analysis systems are met.

The second area receiving attention is refinement of the image processing methods, and the pattern matching algorithms. Once the images are acquired, techniques must be applied to extract the relevant components from the whole. From coarsest to finest, the relevant components are:

1) The fish, as separate from any background.
2) From the fish, features of the head must be identified:
   a) Nares
   b) Head length
   c) Spots

Once the fish is separated from the background, specific relevant features are identified in order to define the area of interest for pattern extraction. Below are two unmodified (except for cropping), typical images selected at random from the hatchery images. In them the nares and the anterior and posterior margins of the head are easily identified. Also note the detail in the eyes.

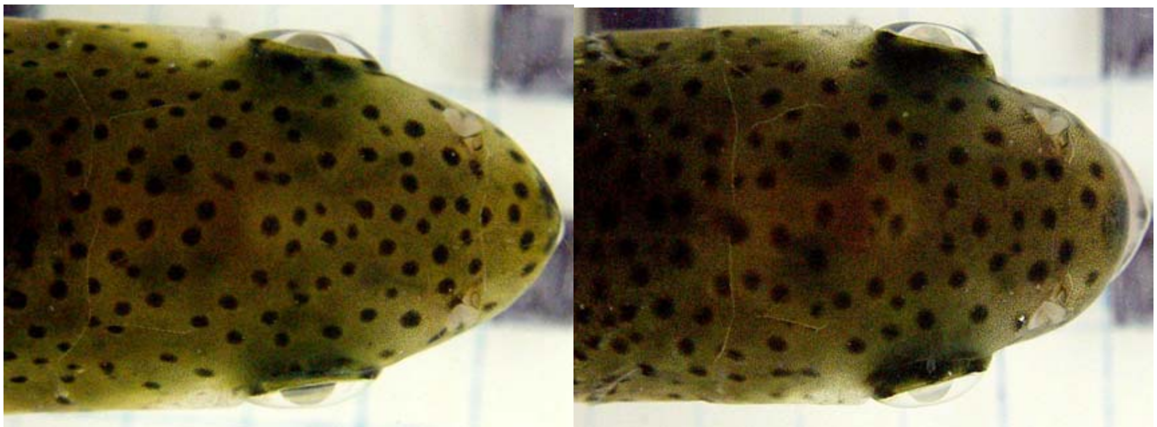

Figure 13.

Once the relevant dimensions of the head are extracted from the image, an area-of-interest determined by those dimensions is selected for specific processing to create a mask to threshold the image and render a binary image for pattern matching. Using the images above, the resultant binary images are presented below.

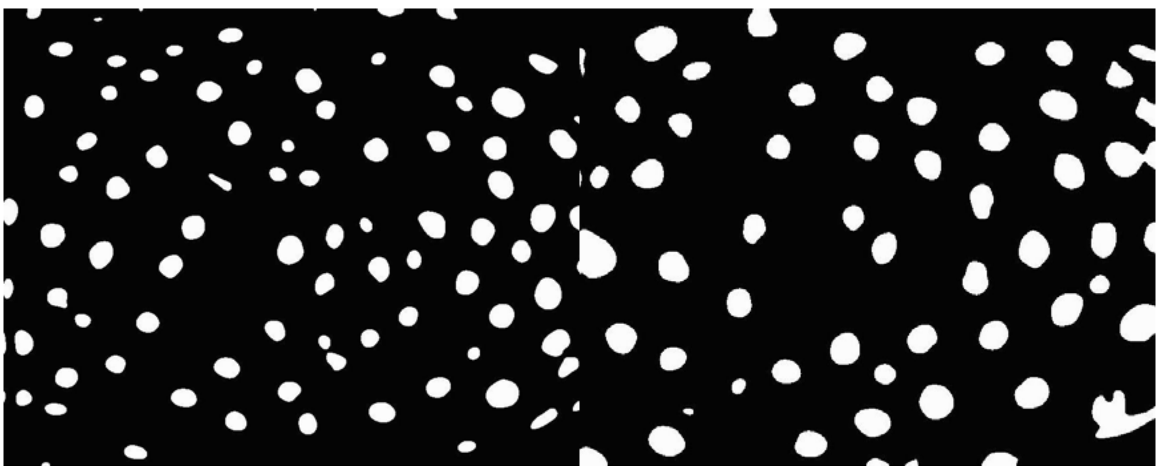

Figure 14.

As may be seen, there are "spots" of various sizes and shapes in each of these images. Selecting which of them are true melanophore-derived spots can be challenging. (The oddly shaped spot in the lower right of the right hand image is part of the nares for example.) Implementation of color-based filtering substantially improves the ability of TNT to recognize "true" spots and therefore improves accuracy in a fully automated mode. Furthermore, pre-filtering in the color domain enhance TNT's fully automated abilities.

An integrated image acquisition system consisting of camera, stand, platen, laptop computer and image handling software is not required for successful employment of TNT in projects having annual sample sizes of less than 1000.

TNT can be very effective, particularly in non-fish taxa, with simple use of a quality digital camera with a flash attachment. However, costs for the customer will be higher, because the amount of human-involved processing required for "non-standard' images will go up. The less automated the process the more labor required, with an associated increase in cost.

For the non-smolt, fish IAS, refinement of the prototype is significantly more complex. There are three primary goals of the operational model design: 1) Ease of use, 2) Improved image quality, and 3) Provide 3-space information about the photographic subject.

From a customer perspective, the IAS should be as easy to use as possible. As configured, the prototype IAS is very easy to set up and use, but it does not *easily* acquire high quality images. Experience and above average photographic skills are required to take images that facilitate fully automated processing. It would be a substantial burden to operation if the IAS required extensive training and experience to use. Therefore, design of the operational model is critical. To facilitate ease of use and acquisition of quality images, two basic factors will be addressed; position and lighting.

If the IAS is capable of determining the 3-axis position of the subject, then the operator will not be required to be skilled or particularly attentive when placing the fish on the platen. If exact positioning isn't critical, fish can be handled faster. Faster handling means more samples per unit time and less handling stress. To get 3-axis position information, two, nine-laser arrays are projected into the image frame. One array from the side projects a grid onto the side of the fish's head and provides roll and yaw information. The positional distortion of the nine elements is trivially quantified and the necessary adjustment in post processing is easily accomplished. The second array is projected from the camera from a device similar to a ring flash. This array provides pitch information. In the same fashion as the lateral array, post processing makes any necessary adjustment easy. By implementing these arrays, the operator simply has to coarsely align the fish with the visible laser arrays and take the picture. Post processing will handle the rest. In addition to facilitating ease of use and determining 3-axis position, these superimposed grids will provide quantitative information about the curvature of the fish. Using that information, the fish can be 'unwrapped' to a flat surface. This may very well lead to greater discriminatory capabilities and therefore greater speed and accuracy.

In conclusion:

All of the goals and tasks presented in the Phase I proposal were accomplished. Of specific importance is the development of a working prototype capable of accurately (100%) identifying juvenile *O. mykiss* over an interval of time (more specifically growth) that represents the greatest growth rate of their life cycle. Ultimately, TNT needs to work in a fully unsupervised mode. In order to realize the same 100% accuracy in an unsupervised mode, (currently 91% in a fully unsupervised mode) the only requirement is non-blurred, non-occluded (bubbles or specular reflections) images. Acquiring images of such quality is not difficult.

What is also now clear that was not apparent at the outset is that the computationally intensive image-processing and pattern-recognition tasks are far beyond the laptop and desk-top computing platform. Not because of lack of computing power *per se*, but because of the enormous number of images that ultimately will need to be processed.

Major benefits of TNT are:

- A non-mutilating method of uniquely identifying individual animals, requiring less handling and lowered mortality.
- A method of identification that stays with the animal throughout its life, allowing multiple recaptures without harm, as well as performing automated morphometric data recording and analysis.
- As a non-invasive method, TNT offers the answer to care-in-handling and related mandates of the Endangered Species Act of 1973, as well as regulations promulgated by the Secretary of Agriculture pursuant to the Animal Welfare Act.
- TNT permits considerably more animals to be "marked" per unit effort. Considering the most commonly used current methods for fish, the cost-per-recovered-fish decreases by at least 50%, and in some cases is reduced by 90%+.
- Considering that a complete statistical report including population estimate as well as morphometric and geographic data is available in as little as three days, "turnaround time" is much faster than some methods, and no slower than all methods currently used.
- A pictorial and statistical history of the animal is created and maintained in a globally available database. Large geographic areas, including multiple species and transcending whole eco-systems would allow system-wide quantitative and qualitative analysis. Post-mortem images of hard structures like otoliths and bone cross-sections, available only via lethal sampling, as well as images of non-lethally obtained structures like scales and fin-ray cross-sections, could be linked to the living-fish images and data in this global database.