Triploid Trout In Native Trout Waters: Phase 1

Literature Review and Recommendations for Phase 2

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This report consists of a literature review on all-female triploid brown trout and gives recommendations for carrying out phase 2 of the project. It will be of interest to Fisheries staff and scientists both within and outside the Agency when considering stocking these fish.

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EXECUTIVE SUMMARY

This report has been prepared in support of the Environment Agency's National Trout and Grayling Fisheries Strategy and its associated stocking policy. It presents the results of four tasks: a review of the triploiding process and biology of all-female triploid brown trout; a review of commercial triploiding practices; an assessment of the feasibility of large-scale production of triploid brown trout in England and Wales, and design of future investigations comparing fishery performance and impacts of stocked diploid and triploid brown trout on wild brown trout stocks (Phase 2 of the Environment Agency project). The outcome of this project will inform a review scheduled to take place in 2006 to assess whether further restrictions on the stocking of farm-bred diploid trout (exclusive of those bred from local wild brood stock) and their substitution by triploid brown trout are justified.

The literature review covered the scientific and technical advances and effectiveness of the processes used to produce sterile triploid trout and the biological characteristics of triploid brown trout, and was combined with information obtained from interviews with other researchers, farmers currently producing triploid trout (both brown and rainbow) in the UK and others who have experience of the performance of triploids in fisheries.

The most reliable way to produce sterile brown trout is through all-female triploids, using milt from masculinised genetic female fish to fertilise normal diploid eggs that are subsequently pressure shocked to interrupt the second meiotic division and restore the maternal diploid component. Whilst this technique has the potential to achieve 100% triploidy induction, a second approach, using sperm from a diploid individual to fertilise eggs from a tetraploid female, appears to be much less reliable and is not recommended.

Though the requisite procedures are well documented for rainbow trout, there is no definitive description in the literature of how to produce triploid brown trout by pressure shocking. A programme of quality control would need to be in place to ensure that the batches of fish produced were 100% triploid.

Little has been published on the biological and fishery performance of triploid brown trout, but observations for other salmonid species have been used to suggest the important differences between diploid and triploid trout.

- No record of a 'female' triploid producing viable eggs has been published.

- Female triploids exhibit similar or slightly lower growth rates, but growth is not depressed by gonad maturation as it is for diploids.

- Triploids are generally more sensitive to disease, but they do not suffer the post-spawning mortality seen in diploids.

- Triploids are less able to cope with low oxygen levels and high water temperatures than diploid fish. This may have implications for triploids stocked in rivers subject to climate change effects and where catch and release is practised.

- When reared together in the same tank, diploids are more aggressive than triploid fish, compete better and exhibit higher growth rates. This suggests that wild diploid trout are more likely to out-compete stocked triploids of the same size, though triploid brown trout may show better over-winter growth and survival than mature diploids, and have the potential to grow larger if uncaught.

- Triploid females do not migrate to spawning grounds and are, therefore, unlikely to disrupt normal spawning behaviour of wild fish.

- The quality of the fish should be at least as good as fish presently caught: flesh colour, flavour and fin condition all are considered better in farmed triploids than diploids and less variable seasonally.

We conclude that there is no evidence that the release of all-female triploid brown trout would adversely affect the genetic integrity of natural stocks of brown trout. Though diploids appear to be more aggressive than triploid trout and compete better in farm conditions, stocked triploids (especially if they are larger than wild fish) will, nevertheless, compete with wild trout for limited food and habitat resources. From the anglers' point-of-view, the behaviour of triploid brown trout may be similar to "normal" stock fish, though they will show better condition in winter and spring and their flesh quality is likely to be less variable seasonally. It is possible that they may show poorer performance at elevated temperatures.

A review of commercial practices, to assess the technical feasibility of producing triploid brown (and rainbow) trout in England and Wales, was based on interviews with trout farmers including some who do not produce triploids in order to understand how they might respond to a policy requiring more extensive production of triploid brown trout. These revealed the techniques used to induce triploidy, including the effectiveness in terms of % triploid and survival at each life stage, together with any special husbandry requirements. Comments were also made about commercial viability in relation to diploid brown and/or triploid and diploid rainbow trout.

Whilst the trout farming industry has successfully produced all-female rainbow trout since the mid 1980s (88 UK farms produced 1833 tonnes of all-female triploid rainbow trout for restocking in 2002), production of all-female diploid and triploid brown trout is a recent innovation, and only 5 farms are currently producing the latter. These farms have developed an effective and commercially viable method for themselves, and are presently unwilling to share this with competitors. Four farms use pressure shocking and report that they have an effective 100% triploidy rate.

The fertility of sex-reversed male brown trout is approximately 80% of that of normal males. High quality ova are needed for the triploid process and, even then, survival of the ova to hatching is usually 80-85% that of a comparable diploid batch. Triploid fry and fingerlings grow more slowly than comparable diploid stocks and appear less competitive, so it is important that triploid batches are kept separate on farms.

The growth advantages associated with triploid trout begin to show after about 16-18 months when diploid fish begin to divert energy into producing gonads. The triploids continue to grow at the same rate and soon overtake the diploids, which never regain this differential.

It is reported that triploid fish are less robust than diploid fish and are more prone to stress, especially reduced oxygen. They require more careful handling, reduced stocking densities and first use of good quality water.

Whilst farmers producing triploid brown and rainbow trout claim to charge a ~ 10% premium to their customers, most felt that this did not cover the increased costs of production.

The increased costs associated with producing all-female triploid trout are difficult to assess. Most are indirect costs: a stock of sex-reversed males must be established, and these and all batches of ova and growing fish must be kept separate and held in the best quality water available. This has a knock-on effect for the rest of the stock and can lead to inefficient use of the holding facilities. The capital cost of the equipment for pressure shocking is between £5 and £7 thousand, plus the costs of researching and designing the equipment, which needs to be purpose made.

Our enquiries indicated that there may be a strong market resistance from customers to the stocking of all-female diploid and all-female triploid brown trout, especially from river anglers who feel that mixed-sex trout will benefit their fishery with increased spawning fish. Though people who are happy to stock with triploids appear to be in the minority, there is a trend in all angling to stock larger fish, especially in commercial still-water fisheries.

The present demand for triploid brown trout appears to be met by the current supply (28.6 tonnes of triploid brown trout were sold for restocking in 2002). Future increases in demand are most likely to result from changes in the Agency's stocking policy.

Examination of the Live Fish Movements Database from May 2001 to October 2003, indicates that approximately 435 tonnes of brown trout (960,000 fish) were stocked into English and Welsh rivers each year. The search also revealed that, of 40 movements from Scotland into the north west of England in 2003, only one involved brown trout (not triploids). We estimate that 380 tonnes of brown trout were produced by farmers for restocking in 2002, and this suggests that most brown trout produced in England and Wales are stocked into rivers. These data must be viewed with caution and, since brown trout are also stocked into stillwaters, it appears that the Agency's Section 30 data may over-estimate the number of fish stocked into rivers. Recommendations are made for additional information to be recorded on the Section 30 application form, including each batch of fish being categorised with respect to both ploidy and sex.

An evaluation of the potential for increases in triploid brown trout production in England and Wales (to meet potential demand under the Agency's Strategy in relation to "native trout" waters.) was based on information collected in the review of commercial practices and from a questionnaire survey of diploid brown trout and triploid rainbow trout producers. A risk analysis is presented of the technical, logistic and economic factors that may constrain more extensive adoption of triploid brown trout production, including demand, start-up costs, level of price premium for triploids compared with increased costs, lack of technical knowledge, concerns over fish health etc, and the potential of each site for producing and/or on-growing triploid brown trout. The likely level of uptake and thus production of triploids has been assessed under different scenarios, and recommendations are given to facilitate increased adoption of the technology of producing triploid brown trout.

Currently, 5 million brown trout eggs are produced, of which 11% are triploid. Increased triploid brown trout production is most likely to be achieved through switching from diploid brown, or rainbow, trout production. In each case, this will result in a 20% decrease in total brown trout egg production due to lower survival or stocking rates. If the five farms currently producing triploid brown trout switch all their current diploid to triploid production, the supply of triploid brown trout eggs could be increased by 50%. A stochastic model was used to estimate the likely mean level of triploid brown trout egg production by current diploid brown trout producers at 1 million eggs. However, there is a high level of uncertainty about the uptake of triploid production, which will largely be decided by a small number of individual farms and whose overall output could be less than 50% of their current diploid brown trout egg production.

Further increases in triploid brown trout egg production could be made through switching from rainbow trout production. These farms have the potential to increase triploid brown trout egg production by over 2000%, though considerable financial incentives would be required. Farms which currently produce only mixed-sex diploid brown (or rainbow) trout may take up to 6 years to switch to triploid production (the time taken to establish an all-female brown trout population).

We conclude that there is considerable potential for increased triploid brown trout production by both current brown and rainbow trout producers in England and Wales, though this may be constrained by a lack of technical knowledge (which is not freely available). Dissemination of the method for triploid production would facilitate uptake of the technology, and more research is required to identify the water temperature and quality parameters required for triploid production. Advance notice of changes in stocking policy, and a campaign to inform angling clubs and others about the benefits of stocking with triploids, would encourage diploid producers to adopt the technology needed to switch to triploid production and ensure market stability.

The final part of this report outlines the design of a programme of investigations to compare biological and fishery performance of diploid and all-female triploid brown trout stocked in rivers, and a means to evaluate objectively the risks of stocking with triploids. The field programme is based on population sampling in a range of fisheries and river types, where all-female triploid brown trout and diploid brown trout are stocked alongside wild trout, using marked or tagged batches of stocked fish, anglers' catch records and electro-fishing.

We have described how the biological characteristics (growth and survival, intra-specific behaviour) of stocked trout and their behaviour in relation to angling can be evaluated, and how this information can be used in a risk assessment to identify and rank those issues that are most likely to influence the Agency's policy on stocking "native trout" fisheries, and thus guide further research in Phase 2 of the project. The outcome will provide a robust and defensible mechanism for decision making in relation to the Agency's National Trout and Grayling Fisheries Strategy.

1 BACKGROUND TO THE PROJECT

The Environment Agency has developed a National Trout and Grayling Fisheries Strategy in which its statutory obligation to maintain, improve and develop fisheries must take cognisance of potential threats when considering applications for Section 30 consent to introduce fish to inland waters. The Strategy recognises that, whilst stocking is crucial to the success of many trout fisheries, there are risks to wild stocks attached to stocking with farm-reared trout. This may occur either because the trout population density is increased, thus creating competition or predation by stock fish and/or stimulating an influx of predators or higher fishing pressure, in both cases leading to higher mortality of wild stocks, or by introduction of disease or effecting a change in the genetic composition of wild stocks through interbreeding.

Within the context of the Strategy, it is considered that the large-scale stocking of rivers and streams with farm-reared fertile brown trout (*Salmo trutta*) may pose an unacceptable risk to wild brown trout populations via genetic change resulting from interbreeding of stocked and wild fish. All-female triploid brown trout have been available commercially for some 5 years and, being essentially sterile, they offer a means of substantially reducing or avoiding genetic risks to wild stocks whilst maintaining the fishery benefits of stocking in "native trout" waters. However, it is possible that their presence in certain types of river, at levels that significantly enhance angling success and the viability of recreational rod-and-line fisheries, may (like any stocked fish) threaten the sustainability of the wild trout populations. Detrimental impacts could include competition for food and habitat resources, predation on wild trout eggs and fry or agonistic behaviour on spawning grounds (Solomon, 2000). The quality of stocked trout, as a sporting adversary and on the table, is also important to anglers.

In support of the National Trout and Grayling Fisheries Strategy and its associated stocking policy, the Environment Agency has initiated a project with the overall objective to examine the production, costs, post-stocking performance and effects on wild stocks of stocked, all-female triploid brown trout. This programme of work addresses the need to understand and compare the impacts on wild trout populations of stocking with farm-bred diploid (usually male and female) and triploid (female only) trout, to evaluate the relative performance of the latter in fisheries, and to assess the viability of large-scale production of all-female triploids to support potential demand (if triploid stocking is found to be preferable to diploid stocking). This research will inform a review scheduled to take place in 2006 to assess whether further restrictions on the stocking of farm-bred diploid trout (exclusive of those bred from local wild brood stock) and their substitution by triploid trout are justified.

The project comprises two phases, the first phase comprising four tasks to satisfy the following objectives: 1) review the triploiding process and biology of all-female triploid brown trout, 2) review commercial triploiding practices, 3) assess the feasibility of large-scale production of triploid brown trout, and 4) propose future investigations relating to fishery performance and impacts on wild stocks. The last task involves the design and costing of a 2.5 year research programme (Phase 2) that would aim to: i) compare the growth rate, condition, survival, fishery performance and angler perception of stocked diploid and triploid brown trout across the range of river and stream types in England and Wales; and ii) evaluate the range, nature and degree of impacts of triploid (as compared with diploid) stocking on wild trout populations. This report covers work carried out by CEFAS for Phase 1 of the Environment Agency project.

2 POLICY BACKGROUND

Genetic changes have been demonstrated in some wild brown trout populations as a result of stocking with hatchery-reared trout (Aurell et al., 2002). Although the impact of several decades of stocking on indigenous trout gene pools remains largely unresolved (Anon, 2000), there is a risk which must be managed following the precautionary principle.

Other than in fish farms, stocking requires the Agency's ('Section 30') consent. In granting consent to stock, the attendant risks should be reduced to an acceptable level, and the Agency has recently developed general policies on fish stocking, especially with non-native fish, reflecting the recommendations of the Salmon & Freshwater Fisheries Legislative Review (MAFF, 2000). The key aspects of the policies that affect stocking with trout are indicated below.

In the past, substantial numbers of large brown trout have been stocked into waters that are nursery areas for wild trout or Atlantic salmon (*Salmo salar*). The policy (15) states that, in considering whether or not to consent a stocking, the Agency will adopt the guiding principles that fish introductions should not be allowed to jeopardise the well-being of naturally established ecosystems; and there should be no overall detriment to the fisheries (stock, habitat or performance) of the donor water or the receiving water, or to the viability of the fish involved in transfer and introduction. In future, it is intended that such stockings will be avoided where there is a significant risk of an adverse impact on wild trout stocks, and that the Agency will develop guidelines to identify limits on the number and size of trout which could be stocked into different types of water without undue risk of a deleterious impact on wild stocks (Policy 16).

Even outside nursery areas, it may be appropriate to restrict the number, size and provenance of fish stocked so as to reduce the risk of any negative impact on wild stocks, either directly or indirectly. With respect to brown trout, therefore, Policy 17 states that the Agency will only consent stocking of hatchery-reared strains into rivers, streams and other unenclosed waters if the genetic composition of the receiving stock is unlikely to be compromised.

Although a non-native species, the rainbow trout (*Onchorhyncus mykiss*) has been widely stocked for over a century in the UK and is the mainstay of most still-water trout fisheries. While it has also been stocked into many rivers, it appears that few self-supporting populations have become established. It is therefore treated differently from other non-native species. Since rainbow trout are readily distinguishable from brown and sea trout (the anadromous form of brown trout), their use for stocking can enable wild trout to be managed separately from stocked fish. However, Policy 19 (rainbow trout into rivers and streams) states that, subject to other constraints, the Agency will permit introductions where there is a history of stocking to sustain a fishery or where the introduction of non-breeding rainbow trout can be clearly demonstrated to be a preferred option for the protection of the genetic integrity of wild brown trout stocks. In all other cases, the Agency will not consent the introduction of rainbow trout into rivers and streams. Furthermore, the Agency will not grant consent to introduce any non-native species of trout or char (*Salvenilus* spp) into rivers, streams and other unenclosed waters (Policy 18).

To help conserve wild stocks and also to enhance the economic benefit derived from them, trout waters will initially be divided into:

- "**native trout":** waters that have significant natural production of trout (Salmo trutta), whether migratory or non-migratory, or from which there is ready access to other waters with such production;

- **"other":** waters that do not have such production or access.

In addition, within "native trout" waters, **"wild fisheries protection zones"** will be designated where stocking will not be consented for one or more of the following reasons:

- local fisheries interests wish to avoid their "wild" fisheries being contaminated with stock fish;

- the wild trout are considered to be "genetically distinct or evolutionarily important";

- the zone contains important nursery or spawning areas for trout and /or salmon, at unacceptable risk from predation/competition by stock fish.

The National Trout and Grayling Fisheries Strategy provides full details of these fishery classifications.

The classification of river stretches containing trout fisheries will involve consultation with local fisheries interests and (where appropriate) conservation interests. . It will determine whether consent to stock will be granted and, if so, the type of fish that may be introduced (Policies 27+28). In "native trout" waters, it is proposed that stocking is acceptable provided the conditions set out below apply. Any trout, including farm strains, may be stocked in "other" waters.

This contract aims to provide an information basis for Policy 28 in which the Agency intends that policy for native trout fisheries will be introduced in two stages:

1) Until 2007: Where stocking is consistent with practice over the past five years, the Agency will not refuse consent to stock fish because of any potential genetic impact, though it will recommend the use of all-female triploid (sterile) brown trout (or rainbows) or, given an appropriate rearing regime, offspring of local, wild brood stock. This allows Phase 2 of the Agency's Triploid Trout project to be conducted.

2) From 2007 onwards: Subject to the outcome of this research and extensive consultation with fishery interests, stocking consent for fisheries will become dependent on compliance with the policy. Apart from considerations about the type of fish stocked into fisheries to avoid the risk of detrimental effects through interbreeding with the wild stocks, considerations such as avoiding stocking of nursery areas in catchments containing native trout or salmon stocks must be taken.

3 TASK 1. REVIEW OF METHODS TO INDUCE TRIPLOID TROUT, AND THEIR CHARACTERISTICS

Project objective: To carry out a review of the scientific literature on the techniques and effectiveness of the triploiding process and the characteristics of triploid brown trout, including consultation with existing producers, users and experts in the field.

3.1 Background and Rationale

Although there is anecdotal evidence from both producers and users that an expansion of the use of all-female triploid brown trout could be received favourably, there is little scientific information on the performance of all-female triploids after stocking, and especially how they interact with wild trout or other species.

Brown trout are routinely stocked into stillwaters and rivers in England and Wales, chiefly for the purpose of providing or enhancing sport for anglers, but also to replace depleted populations following fish kills. A large proportion of these fish are stocked into waters containing a self-sustaining population of wild trout, and there is concern about the possible effects of these introductions on native trout. One solution is to stock with all-female triploid brown trout, which are infertile and would avoid any genetic impact on the wild population. Commercial production of triploid brown trout is now well established, and some fisheries in southern England already choose to stock them, including on the Test and the Allen. It is possible, therefore, that such fish could be made more widely available for stocking in the near future.

Female triploid brown trout look similar to ordinary brown trout but, being infertile, they do not develop eggs or characteristics linked to sexual maturity and there is no evidence that they exhibit spawning behaviour. As a consequence, they are likely to maintain their condition and flesh pigmentation and survive better over winter, and may show better growth rates than ordinary trout because they are not putting their energy into egg production.

A literature review was carried out covering the scientific and technical advances and effectiveness of the processes used to produce sterile triploid trout and the biological characteristics (growth, survival, longevity etc) of triploid brown trout. This has resulted in two deliverables: a succinct review of the available published information which is presented in sections 3.2 to 3.6 below, and an annotated inventory/bibliography of peer reviewed (from ASFA abstracts) and "grey" literature presented at Appendix 1. This section also includes information obtained from interviews with other researchers who have worked in the field, those currently producing triploid trout (both brown and rainbow) in the UK and fishery owners who have experience of the performance of triploids in the fishery (carried out under Task 2).

3.2 Methods for the Production of All-Female Triploids

This section details the technologies for the production of all-female triploid brown trout and their efficacy, and considers areas for the future development of these technologies. All-female stocks of fish are normally produced by fertilizing eggs with sperm from genetic females that have been sex-reversed using hormonal treatment. Triploidy can be induced in two ways: either by providing a physiological shock to the egg during the second meiotic division shortly after fertilization, or by crossing tetraploid individuals with diploids.

3.2.1 Production of sex-reversed male broodstock

Phenotypic, functional male trout can be produced by sex-reversal of genetic females by hormone treatment. Unlike normal genetic males, they are homogametic (the XX state), so all the sperm they produce carry the X sex chromosome and all eggs fertilized by these sperm will develop as genetically female progeny. The method for producing sex-reversed male fish is simple and effective; it involves supplementing the diet of first-feeding fry with 17α methyltestosterone. This is reliably achieved in brown trout by administering the hormone at a rate of 6 mg per kg of feed for the first 1200 day.degrees C of feeding (Lincoln, 1996). The success of the process can be verified by microscopic examination of the gonads of a sample of the treated batch before the fish reach 2g.

If mixed-sex fry are masculinised in this way, the proportion that is genetically male anyway (the heterogametic XY state) would sire mixed-sex progeny upon maturation, as normal. It is straightforward to separate these from the homogametic, XX-masculinised fish, since sperm ducts do not develop properly in sex-reversed males produced by the recommended dose of hormone, and their milt cannot be stripped conventionally. Fish that otherwise appear mature, but are not running, can be selected from the mixed group and checked for incomplete ducts. The testes are then dissected out, minced and the milt suspension used to fertilize eggs, with good rates of fertilization (Bye and Lincoln, 1986). The resulting embryos will all be genetically female.

3.2.2 Production of all-female triploid fish – the meiotic route

In order to produce all-female triploids, eggs are first fertilized with the sperm from a sex-reversed, XX type, male (as described above).

The most common and easily achieved route of triploidy induction involves applying a physiological shock to newly fertilized eggs during the second meiotic division, causing the second polar body to be retained rather than lost. This results in a zygote with three sets (triploid) of chromosomes instead of the normal two (diploid). The shock can be provided by heat or cold, pressure or chemical means. The timing, duration and magnitude of shocks are critical to success rates in inducing triploidy.

Chemical methods

Cytostatic chemicals such as cytochalasin B have been used in attempts to induce polyploidy in some salmonids. Treatment of newly fertilized Atlantic salmon eggs with cytochalasin B carried across the cell membrane by DMSO resulted in diploid/triploid mosaics, sometimes also with tetraploid cells (Allen and Stanley, 1979). Similar treatment of rainbow trout eggs also resulted in production of a variety of ploidies and mosaics. Survival rates were low. Refstie (1981) concluded that tetraploids may have been produced. The poor reliability of inducing triploidy, the hazardous nature of the chemicals used and the availability of more successful, alternative, methods have led to little further work using chemical treatments to induce triploidy in finfish. This approach is, therefore, considered to be inappropriate.

Cold shock

Cold shocks have been used in an attempt to induce triploidy in brook trout (Lemoine and Smith, 1980). Survival of treated embryos was low and mosaic polyploids, but no exclusively triploid fish, were produced. These procedures are considered to be ineffective in salmonids (Lincoln & Bye, 1980).

Heat shock

Arai and Wilkins (1987) reported the results of heat shock trials on brown trout eggs. They found that shocks of 29°C and 1 minute duration, initiated between 5 and 45 minutes after fertilisation, gave high frequencies of triploid embryos (77-91%) as assessed by chromosome examination. Shocks initiated between 90 and 260 minutes following fertilisation produced no polyploids. Other trial groups heat-shocked at 29°C for 5-15 minutes duration initiated 10 min after insemination resulted in moderate rates of triploidy (50-63%). A high temperature shock of 32°C for 6 minutes duration gave 100% triploidy, but a lower temperature shock of 26°C, even for 30 min duration, had only a moderate success rate (57%). Rates of hatching were generally lower in the treatments giving moderate to high frequencies of triploidy.

Quillet *et al.* (1991) reported that rates of triploidy close to 100% could be achieved in brown trout without causing unacceptably high mortality using a heat shock of 10 to 15 minutes at 28°C, starting 5 minutes after fertilization. Subsequently, Moffet and Crozier (1995) found that treatment using a single heat shock of 28° C for 10 minutes, applied 15 minutes after fertilization to five different batches of brown trout ova, produced triploid rates ranging from 22% to 100%. Survival to hatch ranged from 13.5% to 63.9% relative to the untreated diploid controls. Variability was greater between batches than among replicates, suggesting that the effectiveness of a given shock treatment was batch specific. In general, the treatments reduced survival to hatch by some 50%.

Lincoln and Scott (1983) and Anders (1990) found that different strains of rainbow trout gave different yields of triploids following the same heat shock treatment. Even with a large, stable body of heated water, the exact magnitude of the temperature shock received will vary slightly from egg to egg. Egg morphology (e.g. size) can presumably affect exact temperature exposure of the zygote and may be strain and batch specific. Another possible explanation for this variation is that the timing of meiotic events could differ slightly between different strains of the same species.

Thorgaard *et al.* (1995) found that heat shock may cause sperm extrusion after activation, thus interfering with induction of triploidy. The unpredictable rates of triploidy induction and low survival rates resulting from heat shock treatment led to the investigation of hydrostatic pressure as a means of shocking salmonid eggs.

Pressure shock

Pressure treatment has been used to produce triploid salmonids since the early 1980s, and was initially reported to give more consistent conversion rates, but higher mortality, than other treatments (Allen and Myers, 1983). Pressure will be uniform throughout a batch of eggs in a vessel, regardless of their size and position, thus each egg will receive exactly the same shock. Foisil and Chourrout (1992) reported that pressure shock treatments induced a gradual change to triploidy, whereas temperature shocks gave an abrupt change. This suggests that the mechanisms by which they disrupt meiosis II are different. For optimal triploid induction, the timing of the application of the shock after fertilization is later for the pressure shock technique (e.g. compare Quillet *et al.*, 1991 with Brydges and Benfey, 1991).

The initial work to identify the optimum pressure treatment technique for brown trout was carried out by Brydges and Benfey (1991). Their results are reproduced in Table 3.1.

Table 3.1 Survival rates (% relative to controls) and triploid rates and yields of brown trout subjected to various pressure shocks (mean ± standard error)

Treatment Variable	Survival Rate (%)	Triploid Rate (%)	Triploid Yield (%)
Duration of shock *(9500 psi, starting 20 minutes after fertilization)*			
3.5 min	64.6 ± 3.1	83.3 ± 8.8	54.4 ± 8.4
4.5 min	72.5 ± 5.3	96.7 ± 3.3	70.2 ± 6.3
5.5 min	89.5 ± 7.6	96.7 ± 3.3	86.6 ± 8.9
6.5 min	95.9 ± 3.2	96.7 ± 3.3	92.5 ± 1.6
Magnitude of shock *(for 5 min, starting 20 min after fertilization)*			
8500 psi	77.9 ± 28.5	76.7 ± 6.7	61.8 ± 24.8
9500 psi	91.7 ± 6.1	93.3 ± 3.3	85.3 ± 3.6
10500 psi	111.1 ± 4.2	100.0 ± 0.0	111.1 ± 4.2*
* NB Triploid yield was calculated relative to controls. In this instance, slightly higher survival of treated fish than controls and 100% triploidy gave >100% triploid yield			
Starting time of shock *(5 min at 9500 psi)*			
10 min	41.4 ± 10.2	30.0 ± 30.0	9.0 ± 9.0
15 min	31.4 ± 3.6	50.0 ± 15.3	15.4 ± 4.7
25 min	67.8 ± 12.5	100.0 ± 0.0	67.8 ± 12.5
30 min	75.3 ± 4.8	96.7 ± 96.7	72.6 ± 3.1

The results demonstrate that very high levels of triploid induction and survival can be achieved by pressure treatment of brown trout eggs. They also suggest that the optimum conditions were not actually achieved in the course of these experiments, as triploid yield had not yet started decreasing with the longest shock duration and highest magnitude of shock. Nor had the optimum start time in relation to duration and magnitude of shock been identified. The most effective conditions were in the range of 9,500 psi to 10,500 psi applied 20 to 30 minutes after fertilisation. It appears that further work to optimise this process could result in a method giving close to 100% triploidy and relative survival, and provide an assessment of the margin of error within which 100% triploidy would normally be achieved.

Water temperature for incubation should also be optimised. Brydges and Benfey (1991) carried out fertilization and incubation at a temperature of 8.5°C, raised to 10.5°C after 5 weeks. Low water temperatures at stripping and incubation (6-8°C) are reported to give high yields of triploidy in heat-shocked rainbow trout (Diaz *et al.*, 1993). It is possible that this effect is attributable to the higher temperature difference making the shock more effective, without causing mortalities by exceeding the upper limit. Though lower incubation temperatures may have less effect in this way for pressure shock treatments, they will have the effect of slowing down development processes and hence increase the window of opportunity for successful shocking.

Though protocols to induce triploidy in brown trout have been developed using both heat and pressure shocks (Lincoln, 1996, mentions that a reliable protocol for brown trout is in place at Allenbrook trout farm), these are of commercial importance and are confidential.

Screening

The pressure shock technique has been optimised at one commercial farm in England, where triploid rates are reported to be consistently 100% with survival rates very similar to untreated controls (Lincoln, 1996). However, production of triploidy by retention of the second polar body will not always be 100% effective, and the timing of the pressure shock is crucial: deviations may result in greatly reduced success rates. As the relatively small size of the pressure containers used means that many small batches have to be processed one after the other, there is considerable potential for treatment timings to differ slightly in practice.

Representative samples should therefore be screened at the swim up stage to verify triploidy. The simplest test is the microscopic measurement of erythrocytes from the hearts of formalin-fixed fry (Johnstone and Lincoln, 1986). Flow cytometric measurement of erythrocyte DNA content is more reliable and rapid, but requires specialised equipment (Benfey, 1999).

Independent screening may be needed, because if the farmer cannot guarantee 100% triploidy the Agency may ultimately not allow his fish to be stocked into native trout waters. Of course, these fish can still be introduced into "other" waters where there is no significant wild trout breeding, i.e. most stillwaters and some streams, or used for the table. It is worth noting, however, that one of the farmers interviewed claims that using optimised pressure shock treatments on Atlantic salmon and brook trout, every fish he has shocked in the last 10 years and tested has been triploid.

Egg quality

The quality of eggs used will affect survival and, possibly, the efficiency of triploid induction. As in conventional husbandry, eggs from 3-4 year old broodstock which have matured at least once before (2 year-old females may mature, but their eggs are generally not used as they are usually smaller and considered of lower quality) should be used, and these should be fully ripe and of a good size. Any strain-specific differences should also be investigated for brown trout. In addition to incomplete induction of triploidy, there is the potential for triploids to become mixed with diploid fish during grading and on-farm movements. It is impossible to tell them apart visually, and working practices should be adopted to minimise the chances of mixing.

3.2.3 Production of triploids via tetraploid-diploid crosses

Theoretically, 100% triploids can be produced by crossing a tetraploid (4n) individual with a normal diploid. Tetraploid individuals can be produced by inhibition of the first mitotic division following fertilization. This event occurs a few hours after the exclusion of the second polar body and, again, can be prevented by shock treatments. Tetraploids are viable, and produce diploid (2n) gametes. A tetraploid line, once created, could be maintained by conventional breeding between tetraploid females and males, although there is a tendency for the male component of the population to increase (there is more chance of having a Y chromosome if an animal has 4 sets, and a Y chromosome is always expressed). When 2n gametes are fertilized by haploid (1n) gametes from normal diploid fish, triploid zygotes result. Using sperm from 2n sex-reversed males to fertilise eggs from 4n females will guarantee all-female triploid progeny.

Induction of tetraploidy has proved to be much more difficult to achieve than induction of triploidy via the meiotic route. There is considerably more variation in the required shocking procedure between individuals, batches and strains, presumably due to the longer delay from a set point (fertilization) and the brevity of the event.

First attempts to produce tetraploid salmonids (rainbow trout) using heat shocks were disappointing (Thorgaard *et al.*, 1981; Chourrout, 1982), but later investigations using pressure shocks provided more positive results. Heat shocks applied during the prometaphase (circa 3-4 hrs post fertilization) and pressure shocks applied during the metaphase (circa 5-6 hours post fertilization) were both effective (Chourrout, 1984). This suggests that pressure and temperature shocks have a different mechanism of cleavage inhibition. Pressure shock treatment was found to give relatively low survival to hatch, but all viable embryos were tetraploid. Ploidy of embryos or fry can be assessed relatively simply by karyological methods (e.g. Chourrout, 1984).

Only one pilot study has been carried out (at Stirling) into the induction of tetraploidy in brown trout (Myers *et al.*, 1995). Hydrostatic pressure was used (9000 psi) to shock the eggs at intervals between 5h 15 min and 9h post-fertilization at 8.5°C. The ploidy of the resulting fry was assessed by red blood cell volume. The rate of tetraploid induction peaked at 90% when the shock was timed at 3904 minute.degrees C post-fertilization, about 72% of the interval between fertilization and first cleavage. First cleavage occurred at 5415 minute.degrees C post-fertilization. Survival to hatch was low, around 25% to 50% relative to the controls, and the yield of tetraploids was about 16%. Tetraploid fry exhibited poor survival post-hatch.

A considerable amount of investment would be required to develop methods appropriate to brown trout and establish a tetraploid line. This would include optimising the pressure shock treatment, karyotyping the progeny, and maintaining a line of tetraploid broodstock. The timing of the shock relative to the first cleavage interval has been loosely established by Myers *et al.* (1995) for brown trout, and further work on the duration and magnitude of the shock would help to refine the procedure. High survival rates and 100% induction of tetraploidy are not necessary as long as sufficient tetraploids are produced to establish a brood stock.

Myers (1991) compared the performance of triploid rainbow trout derived by tetraploid x diploid (interploid) cross with that of triploids produced by the meiotic route. He described better growth in interploid triploids and lower rates of aborted embryos, but reported lower rates of fertilization using sperm from tetraploid males. Arai (2001) reports that better rates of fertilization were obtained in rainbow trout using sperm from a sex-reversed diploid male to fertilize the eggs of a tetraploid female. Lower rates of fertilization by the sperm of tetraploid males may be a consequence of increased sperm head size. However, tetraploid females produce some unreduced tetraploid oocytes as well as diploid oocytes (Chourrout & Nakayama, 1987).

3.3 Differences Between Triploids and Diploids

There are three basic differences between triploids and diploids. Due to the extra genetic material contained within each cell, triploids are more heterozygous, have fewer and larger cells in most tissues, and gametogenesis is disrupted which normally renders them infertile. Despite these basic biological differences, triploids are very similar to diploids at a whole animal level, with the most obvious differences arising in reproductive physiology and behaviour of female fish in particular.

3.3.1 General physiology

In triploids, both nuclear volume and the cell volume are increased, maintaining the diploid ratio of nuclear to cell cytoplasmic volume. Triploid fish are not generally any larger than diploids as a result of this, but have reduced cell numbers in tissues and organs containing larger cells. These cells have a reduced surface area to volume ratio, which could affect exchange and membrane binding processes. Intracellular distances are increased, which could affect internal processes such as signal transduction. However, these apparent disadvantages may be compensated by reduced energetic demands in maintaining smaller overall areas of cell membrane and associated osmotic and ionic gradients. Increased cell size and or decreased cell number has been described in the brain (Lou & Purdom, 1984), epithelium (Lou & Purdom, 1984), muscle (Greenlee *et al.*, 1995), liver (Powell and Kocan, 1990), erythrocytes (e.g. Lou & Purdom, 1984) and leukocytes (Yamamoto and Iida, 1994) of rainbow trout. Comparable differences in erythrocyte morphology form the basis of tests to differentiate between triploids and diploid brown trout.

There have been several studies on aspects of haematology of triploid fish. The main difference is increased erythrocyte (and proportional decrease in numbers). The haematocrit has generally been found to be the same for diploid and triploid salmonids, although one study found reduced haematocrit in triploid rainbow trout

(Virtanen *et al.*, 1990). Total blood haemoglobin concentration has been reported as being the same for diploids and triploids in brook trout (Stillwell and Benfey, 1994), but most studies have found lower haemoglobin concentrations in triploid salmonids (e.g. Graham *et al.* 1985).

The decreased erythrocyte surface area to volume ratio is likely to reduce the efficiency of oxygen transport. Graham *et al.* (1985) found that the amount of oxygen carried by a given amount of haemoglobin was lower in triploid compared to diploid Atlantic salmon. Another possible consequence of increased erythrocyte size may be impeded blood flow through capillaries. However, cardiac performance of diploid and triploid brown trout has been found to be very similar (Mercier *et al.*, 2001).

There have been a number of studies investigating oxygen consumption and aerobic and anaerobic capacity of triploid fish. Most researchers have found oxygen consumption rates are similar to those of diploid fish (Benfey, 1999). This does not imply that aerobic capacity is necessarily similar for triploids. Despite finding similar rates of oxygen consumption for triploid and diploid rainbow trout, Yamamoto and Iida (1994) demonstrated that, as oxygen levels drop, triploids showed signs of respiratory distress earlier and at higher dissolved oxygen levels than diploids. Critical swimming speeds have been found to be the same for diploids and triploids in brook trout (Stillwell and Benfey, 1996), coho salmon (*O. kisutch*) (Small and Randall, 1989) and brown trout (Altimiras *et al.*, 2002). One study found a quicker elevation of plasma lactate and depletion of liver glycogen during forced swimming in triploid compared to diploid rainbow trout (Virtanen *et al.*, 1990). However, other physiological parameters recorded in this study suggest chronic stress in the triploids prior to the experiment may have been responsible (Benfey, 1999).

It is widely held that triploid fish are less tolerant of poor water quality (e.g. low oxygen, high temperature) than diploid fish of the same species. Naturally occurring triploid ginbuna goldfish (*Carassius auratus langsdorfi*) have a more northerly distribution than diploids in Japan, possibly reflecting their reduced ability to tolerate high water temperatures and associated reduced oxygen levels (Sezaki *et al.*, 1991). Mortalities of triploid rainbow trout in aquaculture facilities in France have been reported during the spring and summer months when water temperature is highest (Altimiras *et al.*, 2002). Reduced survival of triploids compared to diploids has been found in rainbow trout reared at chronic high temperatures (Ojolick *et al.*, 1995), but the reason for this was unclear.

Recent work carried out by Hyndman *et al.* (2003a and 2003b) has shown no difference in the recovery of triploid and diploid brook trout when exercised at 9°C, but significant differences in metabolism were found at elevated water temperatures (18°C). Triploids used more glycogen and less phosphocreatine and had difficulty restoring muscle metabolites to pre-exercise conditions and, consequently, suffered high mortalities in the 4 hours post exercise. Altimiras *et al.* (2002) concluded that a reduction in the factor by which metabolic rate is increased from rest to exercise at higher temperatures in triploid brown trout may contribute to the mortalities observed at 18°C.

Triploid brook and rainbow trout showed the same response to acute stressors (handling and confinement) as diploid fish (Benfey and Biron, 2000). Similar results were obtained for Atlantic salmon (Sadler *et al.*, 2000). Nothing has been published (aside from Ojolick *et al.*, 1995, which has already been discussed) on the effects of chronic stressors on salmonids, despite the abundant anecdotal evidence of lower survival of triploids in poor quality water. Lower tolerance of environmental extremes may have deleterious consequences for stocked triploid trout in some fisheries during the summer months.

Osmoregulatory ability in salmonids is apparently unaffected by triploidy, as demonstrated by seawater challenge tests on coho salmon (Johnson *et al.*, 1986), rainbow trout (Quillet *et al.*, 1987) and Atlantic salmon (Jungalwalla, 1991). Long-term survival in seawater was lower for triploid Atlantic salmon, although this has not been attributed to osmoregulatory difficulties. It has been suggested that they require to be a larger size than diploids for successful transfer to salt water (Galbreath and Thoorgard, 1995).

3.3.2 Development and growth

Despite the increased heterozygosity and cell size of triploid fish, rates of development are similar to diploids (Benfey, 1999). For salmonids, triploid rainbow trout were found to hatch slightly earlier than diploids (Quillet *et al.*, 1988) and Johnstone *et al.* (1991) found a slight delay in the initiation of feeding in triploid Atlantic salmon. Higher mortality rates were found for heat-shocked rainbow trout eggs, and for eggs fertilized with sperm from masculinised females (Tabata *et al.* 1997). As well as increased mortalities in triploid rainbow trout eggs, Stevenson (1991) reported a higher incidence of deformities in triploid fry. Madsen *et al.* (2000) noted an increased incidence of spinal deformities among all-female triploid rainbow trout fry.

The growth performance of triploid rainbow trout has been well documented, but there is considerably less information available for brown trout. Generally, female triploid salmonids exhibit similar or slightly lower growth rates compared to diploids, until diploids start maturing. Growth rate, food conversion and protein efficiency ratios of triploid rainbow trout did not differ (0+ juveniles) or were slightly lower (1+ juveniles) than the diploid control (Olivia-Teles and Kaushik, 1990). When both ploidies are reared together in the same tank, the more aggressive diploids compete better and exhibit higher growth rates (Lincoln and Bye, 1984).

3.3.3 Maturation

Male triploid salmonids do mature and divert energy into the development of normal sized gonads, producing related hormones and secondary sexual characteristics (and, presumably, behaviour), but the development of gametes is disrupted during the very early stages of meiosis (as in triploid females). With diploid females, the increase in gonad size is mainly post meiosis as the primary oocytes become enclosed in follicles which are responsible for steroid synthesis and secretion. These do not develop in triploid females, the ovary generally remains as a string-like structure, and no endocrine changes associated with maturation are observed (Lincoln and Scott, 1984).

Major differences are observed when comparing maturing diploid and triploid females. Due to the energetic requirements of oogenesis, fat deposition is reduced during maturation of diploid females and, as a consequence, they have smaller fat deposits around the viscera than triploid females reared under the same conditions (Lincoln and Scott, 1984). Somatic growth continues in female triploid trout during this period and they exhibit better growth rates and no post-spawning mortality. The recent ban on the use of malachite green has resulted in increased mortality of maturing brown trout in culture from *Saprolegnia* infections. However, this problem is not encountered with all-female triploids (Solomon, 2003), and they might therefore be used to advantage for the production of larger fish. In addition, female triploids do not appear to assume behavioural changes associated with spawning and may have better over-winter survival in fisheries.

3.3.4 Immunocompetence

Triploid rainbow trout demonstrate a similar level of immunocompetence to diploids and are equally as responsive to vaccination (Yamamoto and Iida, 1995a, b & c). They are, however, more susceptible to mortalities associated with bacterial gill disease (Yamamoto & Iida, 1994), though this may be a consequence of their haematology. Ojolick *et al.* (1995) reported higher mortalities among triploids than diploids exposed to *vibriosis* in conditions of chronic high temperature stress. During the spawning season, female triploids maintain higher levels of complement activity than diploid rainbow trout (Yamamoto and Iida, 1995c) which, as a consequence, suffer higher post-spawning mortality.

3.3.5 Sensory perception and behaviour

The reduced number of larger cells may affect the sensory perception of triploids. One obvious area where this may have an effect is in the light-sensitive cells in the retina, giving less visual acuity. Aliah *et al.* (1990) conducted experiments designed to assess the sensory perception of triploid fish, and their results suggest that triploid ayu (*Plecoglossus altivelis*) are less responsive than diploids to both sound and light. Deeley and Benfey (1995) found no difference in the number of days taken to learn to avoid an electric shock in a Y-shaped maze, or in the minimum voltage necessary to elicit a response between diploid and triploid brook trout.

Several studies have found triploids to be less aggressive than diploids (Benfey, 1999), which may explain the poorer growth of triploids when reared in competition with diploids. This may be due to differences in the nervous system, or reduced levels of androgens.

3.3.6 Flesh quality and appearance

Muscle fibre distributions were significantly different between triploids and diploids in rainbow trout <300 mm, with a higher proportion of smaller fibres in diploids (Suresh and Sheehan, 1998). Larger hyperplastic fibres in triploids are probably due to the combined effect of increased nuclear size in triploids and the relatively high nucleus:cell ratio observed in small muscle fibres. These larger fibres may be less favourable to cellular metabolic exchange because of their smaller surface area to volume ratios, and this could account for reduced viability and growth observed in triploids during early life stages. For fish >300mm, no difference was evident, suggesting that triploid trout may have higher rates of new fibre recruitment and growth capacity at these sizes. In a blind taste test, a significant preference for triploid fish was found (Bye & Lincoln 1986). No deterioration of the flesh over the spawning period will occur in triploid females.

Juvenile diploid and triploid rainbow trout exhibit a similar ability to fix canthaxanthin (Choubert and Blanc, 1985). Because canthaxanthin is preferentially deposited into the eggs in maturing diploid females, triploid females exhibit better muscle coloration during the egg development period (Choubert and Blanc, 1989). Post stocking, the diet is no longer supplemented with pigments. Flesh colour will deteriorate considerably faster in maturing diploid fish than triploid fish, and may result in a marked difference in flesh colour of over-wintered stocked trout.

It has been reported that triploid rainbow trout regenerate amputated fins more quickly and reliably (Alonso *et al.*, 2000). As fin erosion is common in cultured trout, this may result in stocked triploid brown trout having a better overall appearance.

3.4 Post-Stocking Performance

It is apparent from the above that there is little overall difference between diploid and triploid trout in culture situations, apart from benefits associated with non-maturation. As well as there being no direct genetic interaction with native stocks, post-stocking implications of differences discussed in the previous section infer;

- No disruption of wild trout spawning (e.g. overcutting redds) if female triploids do not develop endocrine-mediated spawning behaviour.
- Probable better over-winter growth and survival.
- Better flesh quality all year round, and possibly appearance (no deterioration associated with spawning, better fin regeneration).
- Triploids probably have the potential to grow larger if uncaught.

However;

- Using the meiotic method of triploid production, 100% triploidy cannot be guaranteed.
- Triploids may be outcompeted by wild fish of the same size due to poorer senses and reduced aggressiveness. (Note, however, that wild fish will probably be better adapted to their environment than cultured fish, irrespective of ploidy).
- Possible poorer performance at elevated temperatures (reduced feeding and hence catchability, increased stress and mortalities at temperatures approaching 20 °C).
- Possibly reduced fighting ability and longer recovery period (for catch and release fish) due to differences in blood and muscle.

No peer-reviewed publications pertaining to fishery performance of triploid brown trout were found in the literature survey. The post-stocking performance of triploid rainbow trout was investigated by Dillon *et al.* (2000). Equal numbers of marked mixed-sex triploid and diploid fish were stocked into streams in Idaho. Of the 5400 of each ploidy released, 17.2% of triploids and 17.0% of diploids were recaptured by anglers. Most were captured during the year of release, but a small number were caught the following year (29 diploids and 23 triploids). It should be noted that the triploids were mixed sex, and a highly domesticated strain of fish was used.

Simon (1993) reports that the growth performance of mixed-sex diploid rainbow trout in South Dakota ponds was better than for mixed-sex triploids. Catch rates were lower for triploids, suggesting lower survival (though this could have been lower catchability), but the data are not available to be sure of the more important factor.

Warrilow *et al.* (1997) stocked yearling mixed-sex diploid and triploid brook trout into a lake with a trap fitted on the outlet. During the spawning season, only triploid males and diploids of both sexes were caught emigrating from the lake towards spawning habitat. This confirmed that triploid male brook trout develop spawning behaviour, but triploid females do not.

Solomon (2003) reports anecdotal evidence of good over-winter survival and growth of all-female triploids stocked into the river Itchen. The fishery manager and anglers felt that the over-wintered triploid fish were more catchable than recently stocked fish at the beginning of the season, and noted that the fish were in good condition. Observations suggested that the triploid fish remained at their feeding stations throughout the spawning season rather than moving to spawning areas.

3.5 Areas for Further Work

A number of aspects of the post-stocking performance of triploid brown trout require investigation. Also, if the meiotic route of triploidy induction is to be optimised and used, a thorough investigation of its effectiveness should be carried out, and the important areas for quality control established. If interploid triploid production were to become the method of choice, work is needed to establish methods of tetraploid induction, broodstock husbandry and breeding.

3.6 Summary of the Review

3.6.1 Methods of all-female production

Augmentation of natural stocks with sterile brown trout requires a reliable means of generating batches of infertile, all-female triploid fish, without contamination by diploid individuals. No record of a 'female' triploid producing viable eggs has been published.

The production of all-female triploids requires the use of milt from a masculinised genetic female to fertilise normal eggs before triploidy is induced. The procedures required for the production of these so called sex-reversed males and, subsequently, the all-female progeny are well documented for rainbow trout and have proved reliable over the years they have been in use with brown trout. The techniques are well understood by the industry, but it can be difficult to discriminate between masculinised genetic females and genetic males if stocks of mixed genetic sex are used. The safest approach that would avoid any chance of a genetic male being used in the process by mistake would be only to use stocks of genetically female fish for masculinisation.

3.6.2 Triploid induction

The scientific literature indicates that only two of the published techniques have the potential to reliably achieve 100% triploidy induction amongst a treated batch of embryos: either using sperm from a diploid individual to fertilise eggs from a tetraploid female, or using pressure shock to interrupt the second meiotic division in the egg after fertilisation.

Theoretically a diploid x tetraploid (interploid) cross should give 100% triploid induction, but it appears that production of viable tetraploids (mainly in rainbow trout and Atlantic salmon) is not straightforward, with fish frequently showing mosaicism and aneuploidy that leads to poor viability. The work undertaken to produce triploid rainbow trout this way does appear to have been successful, but the technique has only been used to practical purpose in a research laboratory in Japan. It does not appear to be sensible to attempt to adopt this system in the next few years as a means of producing triploid brown trout in the UK.

Of the other options, the evidence from the literature demonstrates that only pressure shock, when correctly applied, can consistently give 100% triploid induction. It seems that this technique offers the reliability and the practicability to make it the option of choice for producing triploid brown trout, though it would require adequate quality control. The particular pressure used to interrupt meiosis is critical to the effectiveness of the procedure, so the equipment must be well maintained and calibrated to ensure that the required pressure is achieved. Even so, the timing and duration of shock may vary between batches through operator error. To allow for this, the procedure should entail sampling and testing the batches produced to confirm that their triploid status is complete.

The currently available published information about the conditions required for brown trout triploid induction does not reflect the more optimised situation reportedly now in use at several UK farms. The precise pressures and timings used are of commercial advantage to the businesses involved and are unlikely to be made available to third parties without some inducement. This could include licensing and payment for the use of reliable protocols at sites other than those already producing triploids. Alternatively, a programme of research may be needed to determine and specify the conditions that should be used in order to produce 100% triploid stock.

In the course of preparing this report, the views of both Dr. Ray Johnstone (Marine Laboratory, Aberdeen) and Dr Tillmann Benfey (University of New Brunswick, Fredricton) were sought. They were asked for their opinion of the reliability of pressure shock for triploid induction and the potential of the interploid route. Both have worked with Atlantic salmon over many years, and Benfey has also applied the techniques to brook trout. Johnstone did not believe that diploids would ever contaminate batches of triploids produced by pressure shock provided that the apparatus had been properly maintained and was used with the correct pressure and timing. Benfey reported that in their work over the last 10 years, using optimised pressure shock treatment, none of the salmonids tested had been anything other than triploid. As a consequence both felt that the interploid route offered no real advantage over the pressure shock method as long as the risk of diploids contaminating of groups of triploids generated by pressure shock was mitigated by quality control.

3.6.3 Performance of triploids in farm conditions

Little has been published on the performance of triploid brown trout, and we have used observations for other salmonid species to suggest the characteristics that might apply to triploid brown trout. The important differences between diploids and triploid salmonids can be summarised as follows:

Physiology

Generally, female triploids exhibit similar or slightly lower growth rates compared to diploids, but growth is not depressed by gonadal maturation as it is for diploids.

There is evidence that triploids are generally more sensitive to disease, but they do not suffer the mortality seen in diploids that is associated with the post spawning period.

There is evidence that when triploid and diploid fish are reared together in the same tank, diploids are more aggressive, compete better and exhibit higher growth rates. These effects could be less pronounced in natural waters stocked at lower densities.

Triploids are less able to cope with low oxygen levels and high water temperatures than diploid fish, but this too is unlikely to be a significant disadvantage in the rivers where they would normally be stocked (though note possibility of climate change effects).

Interaction with wild fish and fishery performance

There is no information in the scientific literature on the performance of triploid brown trout after stocking. The limited evidence available for species such as rainbow and brook trout has been used to indicate the situation for brown trout.

Triploid female brook trout, unlike triploid males, do not migrate to spawning grounds, reinforcing the assumption that the sterile fish do not develop spawning behaviour. They are, therefore, unlikely to disrupt normal spawning behaviour of wild fish. There is no published information on the propensity for triploids (male or female) to display other migratory characteristics, including anadromy.

Growth performance of mixed-sex diploid rainbow trout in ponds is better than that of mixed-sex triploids, confirming evidence from tank experiments and indicating that wild diploid trout are more likely to out-compete stocked triploids in situations where the food supply is limiting. Though there is some evidence that mixed-sex triploid rainbows over winter less well than diploids (spawning in spring), triploid brown trout may show better over-winter growth and survival than mature mixed-sex brown trout (spawning in autumn/winter). Triploids may also have the potential to grow larger if uncaught.

Limited data indicates that capture rates of triploid rainbow trout by anglers appears to be no different from those of diploids. The quality of the fish should be at least as good as fish presently caught: flesh colour, flavour and fin condition all are considered better in farmed triploids than diploids and less variable seasonally. However, triploids may be outcompeted by wild fish of the same size due to poorer senses and reduced aggressiveness, and they may show poorer performance at elevated temperatures (reduced catchability and increased mortalities). It is possible that they have reduced fighting ability and longer recovery period (for catch and release fish).

3.7 Conclusion

The production of all-female triploid brown trout could be reliably achieved using milt from masculinised genetic female fish to fertilise normal diploid eggs that are subsequently pressure shocked to restore the maternal diploid component. A programme of quality control would need to be in place to ensure that the procedures were being carried out effectively and that the batches of fish produced were 100% triploid.

There is no evidence from the literature to suggest that the release of all-female triploid brown trout would adversely affect the genetic integrity of natural stocks of brown trout. Though there is evidence that diploids are more aggressive than triploid trout and compete better in farm conditions, stocked triploids (especially if they are larger than wild fish) will compete with wild trout for limited food and habitat resources.

From the anglers' point-of-view, the behaviour of triploid brown trout may be similar to "normal" stock fish, though they will show better condition in winter and spring and their flesh quality is likely to be better and less variable seasonally. It is possible that they may show poorer performance at elevated temperatures.

4 TASK 2. REVIEW OF COMMERCIAL PRACTICES

Project objective: To assess the effectiveness and costs and viability of current commercial practices for producing all-female triploid brown trout and rainbow trout.

4.1 Approach

This section provides an assessment of the technical feasibility of producing triploid brown (and rainbow) trout in England and Wales, based on interviews conducted by visits and email or 'phone contact, using a structured set of questions designed to derive specific answers on:

- techniques used to induce triploidy, including broodstock treatments;
- effectiveness in terms of % triploid and survival at each life stage;
- any special husbandry precautions/benefits/problems;
- indicative costs compared to diploid all-females;
- commercial viability in relation to diploid brown and/or triploid and diploid rainbow trout.

For Tasks 2 and 3, we contacted commercial trout farmers currently producing triploid brown trout in England and Wales and others producing triploid rainbow trout, and also interviewed some farmers who do not produce triploids in order to understand how they might respond to a policy requiring more extensive production of triploid brown trout. The scale of the operation was taken into account in relation to the availability of brood stock, the triploid process and rearing-on. In the following review, we have presented information based on observations made by at least three of the five main producers of triploid brown trout, included as little interpretation as possible, and attempted to preserve commercial confidentiality. Note that an assessment of the effectiveness and costs of current commercial practices for producing all-female triploid brown trout and rainbow trout can only be achieved through close personal contact with these practitioners.

4.2 Rainbow Trout

The production of all-female rainbow trout is a well-established practice and has been successfully employed by the trout farming industry since the mid 1980s. According to the production figures (Table 4.1) collected by CEFAS's Fish Health Inspectorate (FHI) for 2002, there were 12 farms producing all-female triploid rainbow trout ova. Ten of these 12 farms are now using pressure shocking techniques, and the remaining 2 farms are still using temperature shocking and at present have no plans to change.

All the farms carry out their own assessments of the rates of triploidy, by removing the ovaries of a batch sample once the fry reach 5g in weight and examining them under a microscope. This technique appears to be well established in the industry and is taught and undertaken for farmers by the fish health advisors of the major feed companies. All the farms reported achieving rates of triploidy in their fish of in excess of 90%, with many claiming to have achieved a triploidy rate of 100%. We note that independent validation of such assessments may be required under the Agency's policy.

In 2002, 88 farms produced 1833 tonnes of all-female triploid rainbow trout for restocking throughout the United Kingdom. These fish are produced from both the home-produced ova and from ova imported from a number of international sources. The triploidy rates for the imported ova are unavailable, but there appear to be few complaints from the industry, which leaves us to believe that the rates must be high.

Table 4.1 Summary statistics of trout production in England and Wales 2002

a) **Rainbow trout**

Number of ova produced in E&W ('000s)				Number of ova imported ('000s)				Fish sold for restocking (tonnes)			
No. of farms	All-female diploid	Triploid	Mixed sex	No. of farms	All-female diploid	Triploid	Mixed sex	No. of farms	All-female diploid	Triploid	Mixed Sex
32	23536.5 (19)	9677.0 (12)	2080.0 (14)	38	14855 (21)	6028 (22)	660 (4)	160	824.0 (72)	1833.17 (88)	225.31 (230

b) **Brown trout**

Number of ova produced in E&W ('000s)				Number of ova imported ('000s)				Fish sold for restocking (tonnes)			
No. of farms	All-female diploid	Triploid	Mixed sex	No. of farms	All-female diploid	Triploid	Mixed sex	No. of farms	All-female diploid	Triploid	Mixed Sex
27	356.5 (5)	567.0 (6)	4040.2 (25)	3			200.0 (3)	81	35.32 (10)	28.58 (13)	316.26 (64)

4.3 Brown Trout

The production of all-female diploid and triploid brown trout is a recent innovation within the UK trout farming industry. According to the production figures collected by the FHI for 2002, the 5 farms that are producing all-female diploid brown trout are also producing all-female triploid brown trout. There are no records of any triploid brown trout ova being imported into England and Wales.

One farm has produced triploid brown trout for 5 years, and the rest have been producing them for between 2 and 4 years. Four farms producing all-female triploid brown trout are using pressure shocking and the remaining farm is using temperature shock. All the farms report that they normally achieve triploidy rates close to 100%, with the farms using pressure stating that they believe that they have an effective 100% triploidy rate. One of the farms did report an 84% rate in his second year and put this down to operator error and as part of the learning exercise for producing all-female triploid brown trout.

There is also one farm that produced mixed-sex triploid brown trout as part of an experiment to see if they could actually produce triploid brown trout using the existing heat shocking equipment which they normally use for rainbow trout. This experiment was successful and they are looking to produce all-female triploid brown trout from 2003 onwards.

One reason for producing both diploid and triploid all-female trout resulted from the phasing out of malachite green as a treatment for fungal infections. Brown trout are particularly susceptible to fungal infections, especially when they start to become sexually mature. The males are the most susceptible and many mature in their first year, unlike the females which mature after 2-3 years. Without an effective anti-fungal treatment, losses can become unsustainable. A more recent driver has been the perceived change in Agency policy to stocking rivers and the probable future implementation of this policy.

There does not appear to be a definitive description in the literature of how to produce triploid brown trout by pressure shocking. It seems that the farms now producing triploid all-female brown trout have developed an effective and commercially viable method for themselves, taking at least two years to develop a fully effective technique by using ideas from various sources and through trial and error. All these farms have had to spend time and money developing there own ‘recipe’ for shocking brown trout ova and are presently unwilling to share this with their competitors in the rest of the industry.

A number of factors have to be taken into account to produce good quality all-female triploid brown trout, and it must be recognised that they have different biological characteristics and behaviour from mixed sex and all-female diploid stocks.

Sex reversal: This is an established technique for rainbow trout and appears to work for brown trout, though one farm reported that he initially had problems in producing sex-reversed males. The problem was never clearly identified and appears to have resolved itself.

Sex-reversed males in both rainbow and brown trout have a reduced fertility of approximately 80% of that of normal males. The sex-reversed males often have a delayed maturation cycle compared with the females, resulting in either ova being over-ripe or the need to use photo period or hormone stimulation to ensure the males mature on time.

Ova to fingerlings: The best quality ova from each stripping need to be selected for the triploid process because the stresses involved can lead to increased mortalities in the ova. The survival of the ova to hatching can vary considerably, from being the same as diploids to as low as 40%, but the norm is 80-85% of a comparable diploid batch.

Deformities of the fry are often perceived to be a problem, but the farmers producing triploid brown trout felt that this was exaggerated. The main link to deformities appears to be inbreeding not triploiding, and regular out-crossing and using multiple cock fish reduces this problem to almost zero.

Triploid fry and fingerlings are slower growing than comparable diploid stocks and appear more gregarious and less competitive in the farm environment. It is also important that all the triploid batches are kept separate and not mixed with all-female or mixed-sex diploid fish because they do not compete well and fail to grow.

Yearlings to stock fish: The growth advantages associated with triploid trout begin to show after about 16-18 months when diploid fish begin to divert energy from growth into producing gonad. The triploids continue to grow at the same rate and soon overtake the diploids, which never regain this differential.

It is reported that triploid fish are less robust than diploid fish and are more prone to stress especially reduced oxygen. They therefore require more careful handling, reduced stocking densities and first use of good quality water. They also appear to need more care in handling because rough handling can lead to an increase in deformities in older fish.

4.4 Costs

The increased costs associated with producing all-female triploid trout are difficult to assess because most of them are indirect costs. A stock of sex-reversed males must be established and kept separate, and all batches of ova and fish must be kept separate and held in the best quality water available. This has a knock-on effect for the rest of the stock, with other fish being held on poorer quality or recirculated water, and can lead to inefficient use of the holding facilities. A lot of the production is for ova and fry/fingerlings to other farms that ongrow and do not or cannot rear mixed-sex trout without malachite.

The selection of the best ova for triploiding has an effect on the rest of the stock being produced on site. The increased staff time needed to be spent on the actual process and with maintaining separate batches also needs to be considered.

The capital cost of the equipment for triploiding, assuming that pressure shocking is used as the most efficient method, is between £5 and £7 thousand. This does not take into account any costs in designing and researching the equipment, which is not readily available and needs to be purpose made.

Whilst farmers producing triploid brown and rainbow trout claim to charge a ~ 10% premium to their customers, most felt that this did not fully cover the increased costs of production. Any higher price could not be justified because there is not a strong demand for all-female diploid or triploid brown trout at present.

Our enquiries of trout farmers indicated that there may be a strong market resistance from customers to the stocking of all-female diploid and all-female triploid brown trout, especially from river anglers. The more traditional fisheries are said to still want mixed-sex fish as they feel that this will benefit their fishery with increased spawning fish; they also tend to be suspicious of these ‘new’ fish. People who have stocked triploids and are happy with them appear to be in the minority. On the other hand, some 5 or 6 years ago there was talk of having year-round brown trout fisheries if they were stocked with triploids. Also, there is a trend in all angling to stock larger fish, especially in commercial still water fisheries where triploids are now an accepted fact of life, if not a necessity.

4.5 Stocking

An initial search of the Live Fish Movements Database (LFMD) from the 1 April 2003, when the option to record fish sex came on line, revealed 1825 entries for movements of either brown or rainbow trout. From this it could be seen that 916 (50%) of these records had no sex recorded against them. The rest broke down as follows:

Diploid	187
Female	3
Triploid	205
Mixed Sex	514

The search also revealed that there are some movements from Scotland into the north west of England, that there are two main sources and of the 40 movements only one involved brown trout. Colleagues in the Scottish Fish Health Inspectorate are presently unaware of any triploid brown trout producers in Scotland.

To further aid analyses of the stocking of trout it may be useful for the Agency to consider expanding the ability to record sex on the Section 30 application form. At present, the form only allows the recording of either diploid or triploid against each application, but not against each batch of fish. It may be useful if the three categories of diploid, triploid and mixed sex can be recorded, as they each have different implications when stocked into fisheries. Also it may be beneficial if sex is recorded against each batch of fish listed on the form instead of as a single entry for the whole application.

5 TASK 3. ASSESSMENT OF FEASIBILITY OF LARGE SCALE PRODUCTION

Objective: To assess the feasibility of large-scale commercial production of all-female triploid brown trout to meet potential demand across England and Wales.

5.1 Approach

Based on the information presented in section 4 (Task 2) on the likelihood of other "new" producers adopting the technology, this section provides an evaluation of the potential for increases in triploid brown trout production at those farms in England and Wales currently producing brown and rainbow trout. For this we used information held by the FHI (presented so sources remain anonymous and data cannot be traced to an individual) and the results of interviews, based on the potential of each site for producing triploid brown trout and for ongrowing these fish on site or at other farms. During phase 2 of this project, this evaluation should be put into the context of the potential demand for triploid trout required under the Environment Agency's Strategy in relation to "native trout" fisheries. For this, it will be necessary to know the proportions of the total annual brown trout stocking requirement in England and Wales, split between still water fisheries or others with no brown trout breeding potential, and those with self-supporting populations of brown trout. Information on who is stocking with triploids and into which fisheries should be available from the Agency's Section 30 consents database (also relevant to programme design at Task 4), but note the comments on the availability of these data given in section 4.5.

A risk analysis is presented of the technical, logistic and economic factors that may constrain more extensive adoption of triploid production, including demand, start-up costs, level of price premium for triploids compared with increased costs, lack of technical knowledge, concerns over fish health etc. These constraints and risks have been identified and ranked through an analysis of farm production dynamics and in discussion with producers. The likely level of uptake and thus production of triploids has been assessed, under different scenarios. The final part of this section contains recommendations to facilitate increased adoption of the technology of producing triploid brown trout.

5.2 Techniques Required for All-female Triploid Brown Trout Production

Farms wishing to produce commercial numbers of triploid brown trout (BT) for re-stocking must be competent in:

- the establishment and maintenance of all-female broodstocks
- the induction of triploidy by heat or, preferably, pressure shocking of ova

Details of these techniques are provided in section 4 (Task 2).

The resources required for triploid trout production are:

1. A stock of all-females and sex-reversed (XX) males

2. Materials for producing androgenised food
    - 17α methyltestosterone
    - Isopropanol
    - Safe (adequately ventilated) drying facilities
    - Suitable storage facility

3. Materials for milt collection
    - Microscope
    - Milt extending solution

4. Shocking equipment
    either
    - Pressure vessel & compressor

    or
    - Thermostatically controlled water bath

5. Competent staff, with the appropriate technical knowledge (on shocking time, duration, incubation temperature)

6. QA assessment [preferably independent] for each farmer's production

5.3 Summary of Constraints to Triploid Brown Trout Production

Equipment and other start-up costs

The cost of manufacturing a pressure vessel and buying a suitable compressor has been estimated at being between £5000 and £7000.

Low or unpredictable premium

Currently, the premium that can be charged for triploid brown trout eggs in a limited market is unlikely to cover the increased costs of production (e.g. increased losses, staff time, equipment) compared with diploid production (data given in section 4).

Access to commercially sensitive technical information

No technical information has been published for inducing triploidy in brown trout. Farmers who are already producing triploid eggs have invested considerable time and money in perfecting the technique and are likely not to want to lose their market lead by giving away this information.

Long lead-in time

The minimum time taken to develop a working stock of wholly homogametic (second generation) males would be 4 years. In practice, however, this could take longer and a realistic lead-in time to commercial production of triploid fish for sale could be in excess of 6 years.

Reduced fertility and high failure rate

The output of viable ova following triploid induction has been estimated by farmers currently in production as being 80-85% of comparable diploid batches, using best quality eggs. If general egg quality is compromised for any reason, or if scaling up production requires inferior ova to be included in the shocking process, then the resulting viable output may be very much reduced. In addition, there is scope for operator error (mistiming shock or supplying the wrong pressure), which may have a massive impact on either the viability of the ova or the success of the triploid induction.

Uncertain market and customer attitudes

There appears presently to be no demand for triploid brown trout beyond current supply. Until the potential market for triploid fish is perceived to have improved, due to a change in current Agency stocking policy or anglers' preference for triploid rather than diploid trout, brown trout buyers may be unwilling to stock triploid fish.

Tank space

Additional space and rearing facilities would be required for maintaining additional lines.

Lack of skilled staff

Competent operators are required to manage the efficient through-put of multiple batches of eggs during the shocking process. This is a complex and challenging task.

5.4 Questionnaire Survey of Diploid Brown Trout and Triploid Rainbow Trout Producers

Seven of the 24 (29%) farms currently producing only mixed-sex brown trout eggs in England and Wales were contacted to assess the factors likely to restrict their entry to the triploid market. These farms collectively accounted for 58% of the total mixed-sex brown trout egg production (3.2 million) in 2002. The remaining farms produce either very low volumes of eggs and/or produce solely for their own needs. The results of this survey are summarised below and in Table 5.1.

- The largest constraint to entering triploid production was identified as being the lack of a market. Closely linked to this was a strong customer resistance; people who buy brown trout for re-stocking specifically want mixed-sex fish because of the perceived stock enhancement benefits (to reproductive capacity).

- Financial considerations were found to be the second most important barrier to adopting triploid technology. The capital cost of start up was of major concern to five farms, with an estimated outlay of £5000-£7000 for a pressure vessel alone. Low or unpredictable premium for triploid over diploid production was highlighted by three farms as being a major constraint. Two stated that there would be no premium to be gained by producing triploid brown trout, as customers would not be prepared to pay extra for fish they did not want. When asked what premium over diploid production they would need to encourage them to adopt triploid technology, one farmer replied he would need a massive financial incentive, which he could not quantify, two quoted figures that would not cover the real cost; three were not able to make an estimate.
- Two farms cited reduced stock performance as a barrier to adopting the technique. Only one farmer reported a lack of skilled staff or people competent to learn the techniques required for pressure shocking. No respondent considered that their lack of technical knowledge or poor access to technical information was likely to be an important issue for them, and no-one cited personal ethical objections to producing triploids.
- Two farmers reported that, based on their own experiences of rearing triploid brown trout, their sites were unsuitable for triploid production. The reasons for this were thought to include variable water quality conditions (particularly low pH and high temperature) which triploids have difficulty in coping with, resulting in high mortality and poor growth compared to diploids. This problem may become more apparent as more farms try to rear triploids for the first time.
- Two farmers questioned the ecological impact of triploid brown trout, voicing concerns that large numbers of predatory fish growing unchecked by maturation may adversely affect recruitment of both wild populations of brown trout and other species.

In general, producers of mixed-sex brown trout consider that there is a place for triploid all-female brown trout in specific markets (fisheries not wanting to have closed seasons) or for specific reasons (farms not wanting to rear male fish for health reasons, i.e. to avoid *Saprolegnia* in the absence of malachite treatment), but the need is not widespread.

Table 5.1 Questionnaire response of seven diploid brown trout (BT) producers

	Farms						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Ever produced all-female BT	no	no	yes	yes	no	yes	no
Ever produced triploid BT (+method)	yes (P)	no	no	yes (P)	no	yes (P)	no
Level of interest*	2	4	1	2	1	5	1
Constraints**							
uncertain / lack of market	0	3	3	3	3	3	3
low / unpredictable premium	0	3	0	3	0	3	0
lack of technical knowledge	0	0	0	0	0	0	0
poor access to technical knowledge	0	0	0	0	0	0	0
lack of capital	3	0	3	3	3	3	0
lack of skilled staff	0	0	0	0	3	0	0
high failure rate	0	3	3	0	3	1	0
ethical objections	0	0	0	0	0	1	0
customer resistance	1	1	0	3	3	3	1
Premium required %	0	20	?	+++	?	5-10	?

*1 (no interest) to 5 (very interested)
**3= mentioned by farmer, 1 = identified as constraint when prompted, 0 = rejected as constraint when prompted
P = pressure shocking

5.5 Ranking constraints to triploid brown trout production

The constraints to production (Table 5.2) have been qualitatively ranked based on the information provided by producers and the reviews of triploid trout production.

Table 5.2 Current constraints to triploid trout production

Constraint	Rank
low demand and consumer resistance	1
start-up costs	2
low profitability	3
high failure rate and low production	4
no freely available definitive protocol for brown trout triploidy	5
lack of skilled staff	6

5.6 Likely Uptake of Triploid Brown Trout Production

Increased triploid brown trout production could come from four sources:
1. increased production by current triploid brown trout producers

2. diploid brown trout producers switching to triploid production
3. triploid rainbow trout producers switching to triploid brown trout production
4. imports

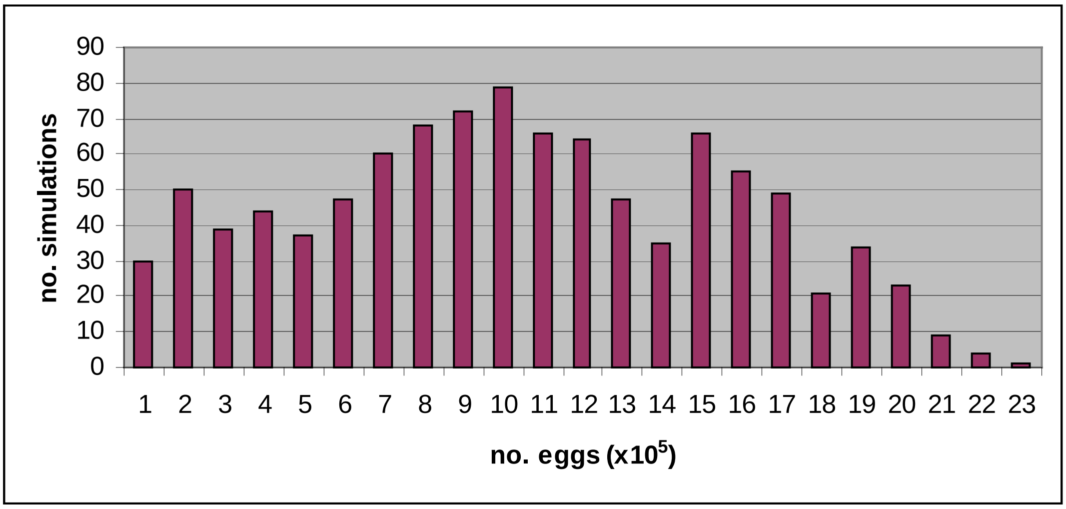
Currently, 4.9 million brown trout eggs are produced, of which 567 thousand (11%) are triploid. It can be assumed that all hatcheries are operating at maximum production, hence increased triploid brown trout production can only be achieved through switching from diploid brown trout, or rainbow trout production. It is likely that a total change from diploid to triploid brown trout egg production will result in a 20% decrease in total brown trout egg production due to lower survival rates of triploids. Similarly, a change from rainbow to triploid brown trout production would also result in a 20% decrease in total egg production (i.e. 80% of the previous rainbow trout production) due to lower stocking rates etc.

The likely level of triploid brown trout egg production by current diploid brown trout producers was estimated using a stochastic model. The @risk software programme (Palisade) was used to construct the model in an Excel spreadsheet (Microsoft). On the basis of the questionnaire survey and other information about diploid BT producers, the predicted level of uptake was estimated to increase with level of production from zero for producers with an annual production of less than 20,000 eggs in 2002 to 50% for the largest producers (>500,000 eggs annually). The probability for each farm was modelled separately, thus a farm within production category 50-100,000 eggs had 25% probability of adopting triploidy for each simulation. The model was run 1000 times. The mean predicted triploid egg production was estimated at 0.99 million eggs, with 90% confidence intervals of 0.15 million to 1.86 million. However, the distribution was not normal (Figure 5.1) and the wide range of values indicates a high level of uncertainty about the uptake of triploid production by diploid brown trout egg producers. Nevertheless, it appears that the potential level of production will in large part be decided by a small number of individual farms.

Table 5.3 Stochastic model for triploid production uptake by diploid brown trout producers

production category ('000 eggs pa)	number of producers	2002 diploid production ('000 eggs)	mean probability of triploid uptake (%)	mean estimated triploid production ('000 eggs)
0-30	9	113	0	
31-50	5	219	15	26
50-100	4	307	25	61
101-500	4	870	33	230
>500	2	1680	50	672
total		3189		989

Figure 5.1 Frequency distribution of predicted triploid egg production (simulations giving each level) by current diploid producers



Farms currently producing triploid brown trout could switch all their current diploid to triploid production relatively easily, since they are all producing all-female stock (scenario 1 in Table 5.4). The only possible constraint may be the capacity of the equipment. A switch by these five farms to triploid-only production would increase the supply of triploid brown trout eggs by 50%. The mean value of predicted triploid egg production from current diploid brown trout producers (0.99 million eggs) has been used in scenario 2. This would result in 174% increase in triploid brown trout egg production. The true figure could be considerably smaller or larger, since it is unlikely that current diploid producers would generate more than 50% of the required increase in triploid egg production.

Further increases in triploid brown trout egg production could be made through switching from rainbow to brown trout production. Five current triploid brown trout egg producers also produce rainbow trout eggs. If these producers switched all their production to triploid brown trout (scenario 3), a 319% increase in triploid brown trout egg production would be achieved. There are a further 6 triploid rainbow trout egg producers who currently do not keep brown trout (scenario 4). A significant increase in profitability would be required for these producers to switch some of their production to triploid brown trout eggs, compared with rainbow trout eggs. Establishing the necessary all-female brown trout population would take 4-6 years (discussed in previous sections). These farms have the potential to increase triploid brown trout egg production by over 2000%.

It is possible that new hatcheries may become established. The investment required would only be justified if good, reliable returns could be reasonably expected. A small volume of diploid brown trout eggs are imported from one supplier in Denmark. To the best of our knowledge there are no overseas sources for triploid brown trout eggs.

Table 5.4 Potential increase in triploid brown trout (BT) egg production under four scenarios

	Scenario	number of producers	annual diploid BT egg production in 2002 ('000)	potential triploid BT egg production[1] ('000)	Percent increase[2]
1	Current BT triploid producers switch all BT production to triploid	6	356	285	50
2	BT diploid producers switching to 100% BT triploid production (based on stochastic model)	24	4040	989	174
3	Current BT triploid producers switch all rainbow trout production to triploid BT	5	2261	1808	319
4	Triploid rainbow trout producers, currently not producing triploid BT, switching all egg production to triploid BT	6	15432	12346[3]	2177

[1]based on 80% diploid BT production
[2]compared with current triploid BT egg production
[3]based on 80% triploid rainbow trout production

5.7 Predicting Future Demand for Triploid Brown Trout Eggs

The present demand for triploid brown trout appears to be met by the current supply. Future increases in demand are most likely to result from changes in stocking policy (e.g. stocking triploids-only in "native" fisheries in rivers containing wild self-sustaining brown trout populations). Though non-triploid producers report customer resistance to triploid brown trout, it is likely that these customers will stock with triploids in the absence of alternatives. Triploids are more expensive to produce than diploids. For the same outlay available to buy brown trout to stock rivers and fisheries, the total number of fish stocked will fall if triploids are sold at a higher price compared with diploids.

Examination of the LFMD shows that, from May 2001 to October 2003, 2832 section 30 consents were approved for brown trout to be stocked into rivers at 1095 'unique' river sites. These consents were for a total of almost 2.4 million fish, broken down as follows: diploid 30,566; female 250; triploids 6,095; mixed sex 112,512. Sex was not recorded for 2.25 million fish, and has been consistently recorded only since May 2003. Assuming an average weight of 0.454 gms (i.e. 1lb) for a brown trout when stocked into a river, this suggests that approximately 435 tonnes (960,000 fish) were stocked each year into rivers. This compares with the 380 tonnes of brown trout we have estimated were produced by farmers for restocking in 2002, and suggests that most brown trout produced in England and Wales are stocked into rivers.

Clearly, these data must be viewed with caution, since there appeared to be some duplicate data entries, not all consented movements will have taken place (Section 30 consents are time limited), or the farm supplying the fish may not have been able to supply the number of fish specified on the application. Over the last 2 years, stocking into rivers (based on section 30 consents) has outstripped production and, since brown trout are also stocked into stillwaters, it appears that the section 30 data may over-estimate the number of fish stocked into rivers.

5.8 Recommendations to Improve Adoption of Triploid Trout Technology

A number of steps can be taken to improve the adoption of brown trout triploidy. We have shown that farms which currently produce only mixed-sex diploid brown trout may take up to 6 years to switch to triploid production. Therefore, it is important that any changes in legislation ("best practice" guidelines are unlikely to have any effect) are made widely known well in advance. The current triploid producers have developed their own systems through experimentation, and adoption of these processes could be facilitated if guidance on an effective system was freely available. Producers currently supplying mixed sex brown trout claim that many customers have a strong preference for such fish. A campaign to inform angling clubs and other about the benefits of stocking with triploids would assist in the uptake of the technology.

5.9 Conclusions

5.9.1 Key constraints

There is considerable potential for increased triploid brown trout production by both current diploid brown trout producers switching to triploid production and rainbow trout farms adopting triploid brown trout production. However, the technical knowledge to implement triploid production is not generally available, and experimentation is required to produce a reliable method of triploidy. Mixed-sex brown trout producers did not consider that technical knowledge would be a serious constraint to adopting the technology, though some may consider (wrongly) that the information is freely available. Dissemination of the method for triploid production would facilitate uptake of the technology. Discussions with some diploid brown trout producers has indicated that some sites have proven unsuitable for triploid production. More research is required to identify the water temperature and quality parameters required for triploid production to better assess this constraint. Whether or not the apparent resistance to triploids among the customers of producers currently supplying mixed sex diploids will continue, if only triploids could be stocked on some or all rivers, will have important implications for assessing future demand.

5.9.2 Assessing a change in demand

A change in legislation that led to stocking with diploid brown trout being banned in certain rivers would inevitably result in an increased demand for triploids and an increase in price if supply does not similarly increase. An increase in price would obviously act as an incentive for producers to enter the market. It is, therefore, important that changes in stocking policy are flagged well in advance so diploid producers have an opportunity to switch to triploid production and to ensure market stability.

5.9.3 Assessing the capacity for scaling up triploid brown trout production

Increases in triploid brown trout production can be achieved most easily and quickly through increased production by the current producers switching diploid brown trout and rainbow trout egg production to triploid brown trout. These producers are best placed to take advantage of an increase in the price of triploid brown trout created by changes in stocking policy. The change in production is likely to occur if there is a profitable and predictable market for triploid brown trout eggs. Currently producers are not able to charge a premium for triploid brown trout that covers the increased costs.

The level of future triploid production by current diploid brown trout egg producers will largely be determined by a small number of producers and is difficult to predict. However, it is clear that production of triploid brown trout eggs will be less than 50% of the current diploid brown trout egg production by these producers. Should it be necessary to achieve a level of triploid production that approaches the current total level of brown trout egg production, it will be necessary for some rainbow trout producers to switch to brown trout production (this assumes the facilities for total egg production remain constant). Rainbow trout producers will require considerable financial incentives to invest in brown trout production, and their start up costs for triploid brown trout egg production will be considerably higher than current brown trout triploid and diploid producers.

5.9.4 Economic assessment

Ultimately, the profitability of producing triploid brown trout, compared with alternative types of fish, will determine the uptake of the technology. The current premium for triploid compared with diploid brown trout is low and is unlikely to attract producers to switch to triploid production. A comprehensive economic assessment is required to more accurately assess the likely change in price of triploid trout given changes in stocking policy, and the impact of price increases on levels of triploid brown trout egg production by different sectors of the industry.

6 TASK 4: FUTURE INVESTIGATIONS RELATING TO FISHERY PERFORMANCE OF TRIPLOIDS AND IMPACTS ON WILD STOCKS

6.1 Project Objective (i):

To design a cost-effective programme of investigations to compare: (a) growth rate, (b) condition, (c) survival, (d) fishery performance and (e) angler perception of stocked diploid and all-female triploid brown trout across the range of river and stream types in England and Wales, making full use of existing stocking programmes.

6.1.1 Fisheries to be used

This section describes the design of a field programme based on population sampling in a range of fisheries and river types, where all-female triploid brown trout and "normal" brown trout (probably both male and female diploid, but this needs to be checked) are known to be stocked. To be cost-effective, it will be important to identify fisheries for which stocking records are kept, both by trout suppliers and fishery managers, and those where participating anglers and management are likely to co-operate in marking batches of stocked fish and recording catch details. We have indicated where options may have different costs (since the budget for Phase 2 cannot be divulged at present).

The Agency's Triploid Trout Project has an overall objective: *to examine the production, costs, post-stocking performance and effects on wild stocks of stocked, all-female triploid brown trout, in support of the National Trout and Grayling Fisheries Strategy and its associated stocking policy*. Bearing this in mind, it is important to ensure that a representative range of fisheries is involved in this research programme. This may mean that river types not currently being stocked with triploid brown trout, but holding self-sustaining populations of wild trout supported (as fisheries) by stocking with diploid farmed fish, may have to be included. In these circumstances, the project might reasonably be expected to fund any additional costs of stocking a batch of triploids as a proportion of the normal stocking regime.

6.1.2 Sampling schemes

The performance of trout in fisheries can be split into two attributes, biological characteristics (essentially, growth and survival, but also including intra-specific behaviour), and behaviour in relation to angling (catchability, and thus return rate and residence time) and quality from an angler's viewpoint. Information on changes in the number of stocked and wild brown trout in a population and estimates of their size (and, therefore, growth and condition) can be obtained by sampling using the semi-quantitative electro-fishing techniques adopted by the EA, for which best practice guidelines are provided in the Agency R&D Technical Summary W2-054/TS (Beaumont et al. 2002) and Health and Safety Codes of practice in NRA (1995). An inventory of existing sampling programmes of riverine salmonid populations that may be used to obtain such samples is available from the EA. Though this is likely to be a costly approach (whoever carries out the sampling), and may only provide snap-shot information on growth, survival and residence time of stocked fish, it may be the only method able to obtain representative samples of wild trout.

The most cost-effective approach with which to measure both sets of attributes, however, is to enlist anglers who visit the stocked fisheries (over the period of the study) to record details of all fish caught. Pawson (1982, 1986) describes a method using anglers' catch data, with stocking records, to estimate the catchability, residence time and growth of trout in a stocked fishery, without distinguishing batches of fish. In order to compare triploid brown trout with those usually stocked, and with wild fish, it will be necessary to use marks that enable stocked fish (both triploid and diploid) to be distinguished.

6.1.3 Trial stocking and marking

The most straight-forward approach to experimental stocking is to introduce marked batches of reared triploid and diploid brown trout, in equal numbers, within the normal stocking regime (size and numbers) of a number of fisheries already stocking with triploids. It is important not to impose "unnatural" conditions in this type of study, and the choice of sites and, possibly, duration of the study will, therefore, depend on stocking and catch levels being sufficient to provide statistically meaningful results.

If stock fish can be reared and selected to a given size (see Pawson & Purdom 1991), then it is unnecessary to give individual fish distinguishing marks. Panjet dye marking (Hart & Pitcher 1969) is benign and has proved sufficiently versatile and durable to enable batches to be recognised for longer than one year, given some training of participating anglers (see Pawson & Purdom, 1986). At the time of the trial stockings, which do not have to cover all fish stocked in any particular year, all triploid brown trout and proportion of similar sized diploid fish can be marked on the belly with a batch-distinct spot/s of dye (Alcian-blue, for example) using a panjet inoculator (see Figure 6.1).

In some fisheries, however, it might be deemed necessary to use the range of fish sizes normally stocked, especially where a proportion is small fish introduced to be released if captured so that they can grow on. In this case, individually distinct tags that can be easily read without sacrificing the fish (e.g. Floy tags) might be needed.

Whatever method is used, it is important to strike a balance between using a type of mark or tag that allows anglers to recognise triploid and other fish, and avoiding undue interference with the conduct of their sport that might lead to changes in anglers' expectations or in their fishing behaviour. If the experimental conditions impose a change in angler behaviour, this will bias the results and make them less applicable to the use of triploids in fisheries in general. It may also result in less than wholehearted involvement of the anglers in the study. Table 6.1 summarises the utility of mark/tag types for this purpose, and their relative costs.

Table 6.1 The utility of mark/tag types for identifying stocked trout in relation to main Phase 2 requirements, and their relative costs

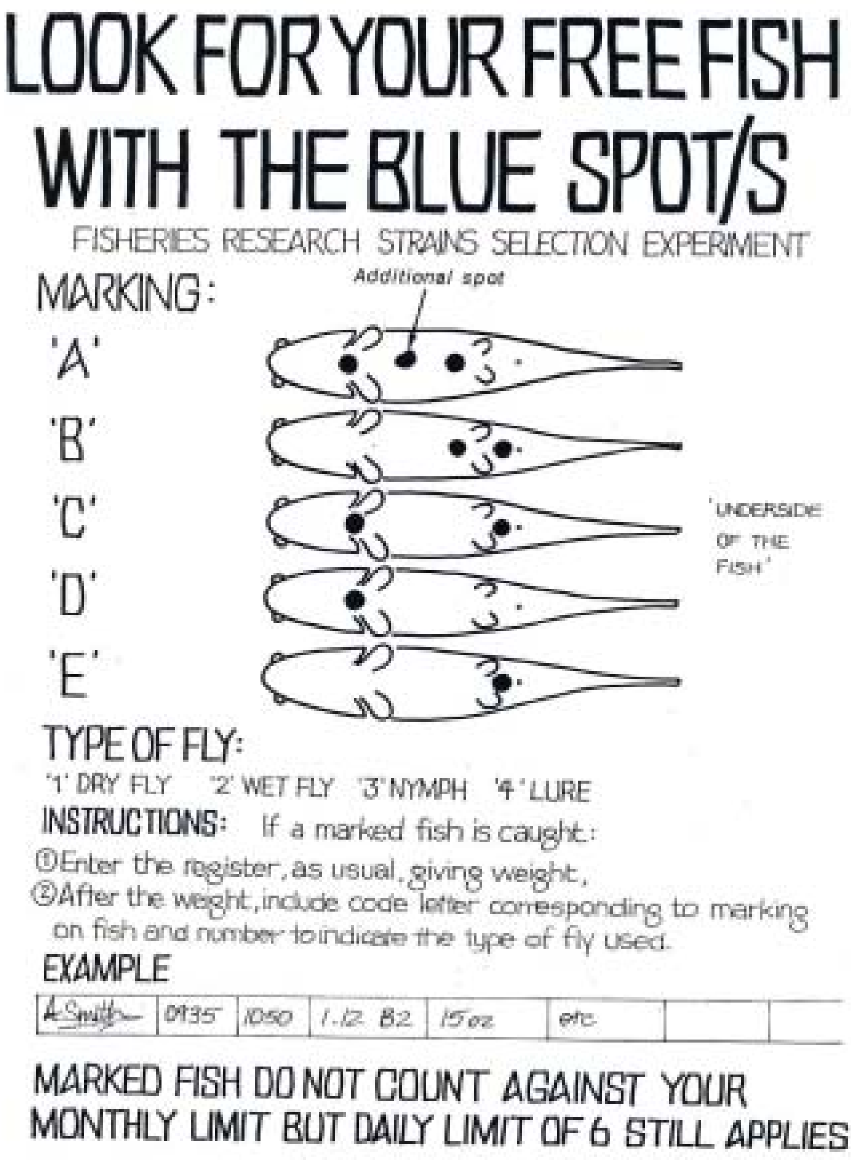
Mark/tag	Benefits/main uses	Disadvantages	Cost level
Dye mark (e.g. by panjet)	easy to apply, easily recognised: (b) (c)	batch marking only	Very low
Eye tag	easy to apply (with training), individual fish: (a) (b) (c)	Needs care and training to identity and record	moderate
External tag (e.g. Floy)	Ditto: (a) (b) (c) (d)	Ditto: may result in targeted fishing/	ditto
Acoustic tag (e.g. PIT)	Can be used to locate as well as identify fish: (c) (d)	More intrusive and skilled application required	high
Radio tag	ditto	Ditto; less precise location	Very high

(a) growth rate,
(b) survival, residence time, catchability
(c) movements
(d) intra-specific behaviour

6.1.4 Catch records

To enhance the collection of scientifically robust data and information needed for this study, it is an advantage to develop and adhere to a protocol for working with angling associations or clubs in monitoring marked fish. Once the likely participating fisheries have been identified, it is essential to arrange a meeting with managers and anglers at each fishery to explain the purpose of the research, how it will be conducted, and what is required of them. In most cases, the ideal is not to interfere with their normal fishery management practices, except that some of the fish stocked will be marked and the anglers will be requested to provide details of all fish caught for the duration of the study. If the fishery already keeps good records (date fished, fish caught, size and species, for each angler visit), then this task is made much easier. Ideally, daily records should be kept of the number and size of fish taken during each fishing season - April-September inclusive, say - and the starting and finishing time of each angler's fishing session. It may be necessary to arrange for the weight and length of dye-marked fish to be checked in order to ensure that high quality growth data are collected. Anglers will additionally be requested to examine their catch and make a note on catch sheets or in the fishery's log of any dye-marked fish. Publicity for the trial should be renewed at the start of each season, even if no further stock fish are dye-marked after the first year. Figure 6.1 illustrates the sort of publicity required.

Figure 6.1 **Poster advertising trout performance trial and showing scope for batch marks**



6.1.5 Angler perception

One option for gaining information on the performance of marked batches, which should be "blind" to the anglers and management, is to ask for anglers' opinions on the "fighting qualities" of the trout, for example, using comments in the daily catch records. In addition, a questionnaire designed to provide information on "angler perception" of the use of triploids could be used on all fisheries currently stocking with them (bearing in mind government strictures on the use of questionnaire surveys), or this could be addressed at an end-of-study briefing at each participating fishery. Informed interpretation of the results of anglers catch records may depend on knowledge about the fishery gained through such meetings.

6.1.6 Growth and condition

The growth and condition of both wild and stocked fish captured by directed sampling or anglers can be estimated from body measurements and (possibly) scale samples, taking into account the residence time of each stocked fish in the fishery, and assuming that each recaptured fish is representative of the batch. Making these estimates, and separating the growth of stocked fish in the farm from that achieved in the fishery, is easier if stocked batches are graded for size (and a few scale samples are taken at stocking). But, in some circumstances, it may be necessary to identify individual fish, measuring them at stocking and at recapture (see 6.1.3 above).

The numbers of fish required to show whether there are significant differences between growth in the fishery of triploid and "normal" stock fish depends on whether stocked batches are graded for size. If so, and assuming that a relatively small proportion will survive long enough to actually demonstrate a change in size (ideally length, since weight can vary considerably after stocking and is less easily measured accurately), minimum batch size will be around 100 fish (for P=0.05). If mixed size fish are stocked, batches will have to be marked so that individual fish are identifiable, and growth rates can be estimated employing the instantaneous rate of growth (G_i):

$$G_i = (L_{t+1} - L_t)/ L_t.\Delta_t^{-1}$$

where L_{t+1} is the length at capture, L_t is the length at stocking and Δ_t is the residence time ([t+1]-t). Although this allows data from fish of different sizes and with varying times at liberty to be used, it may be prudent to group results for fish within particular initial length categories, and a minimum of 200 fish per batch is indicated.

Condition (K) is calculated using $K = W_t.(L_t^3)^{-1}$, on the assumption that growth is isometric in trout.

6.1.7 Survival and residence time

Estimates of the residence time of trout in rivers can be derived from estimates of survival rates of stocked fish, using the model developed by Pawson (1982), for which stocked numbers and dates and week by week catch data are needed. The output can also be used to judge the "benefit" of stocked fish to the fishery (essentially, returns to anglers through time and as a proportion of those stocked) and, from estimates of the changes in the population size of stocked fish and their residence time, the potential extent of their impact on wild trout. Note, again, that if residence time is a matter of weeks in a heavily fished water (or where fish emigrate out of the fishery), many of the risks attending stocking might be irrelevant.

Numerical changes in the population can be monitored by determining the relationship between the decrease in catch per unit of fishing effort as fishing effort (DeLury, 1947) or catch accumulates (e.g. Mottley 1946, Butler & Borgeson 1965), from which indices of catchability and/or mortality can be derived. Implicit in these techniques are the assumptions of negligible mortality other than that due to angling effort, and of an unvarying relationship between catch per effort and stock size. Such assumptions are undoubtedly wrong in river fisheries and they cannot easily be tested, but Pawson's (1982) model was later developed by Pawson & Purdom (1987) to compare performance and growth of three strains of rainbow trout in the same fishery, and by Pawson (1991) to compare performance and residence time of brown and rainbow trout. In these cases, the validity of these assumptions is of minor importance.

The data

The data of most use to this analysis are daily catch and fishing effort statistics, from which weekly summaries are prepared in order to achieve an effective integration of catch rates by anglers of different levels of skill, which would otherwise introduce bias. It is important to have details of the dates and quantities of stocking events in the fishery, and of any catch limits (daily, weekly, monthly) imposed on the anglers. It is also useful to know how much of the fishery is available to the anglers, and whether access to particular parts may be made difficult due to vegetation growth etc. Ideally, the fishing records should also contain details of the size of fish in the anglers' catches.

An example of the information required on the conduct of a fishery:
There is no restriction on the amount of fishing time each of the season members is allowed each week, but all fish caught above 2 lb weight must be killed and not returned to the water, with a daily limit of two fish per angler and a monthly limit of six fish. Most of the fishery is accessible, but the main brown trout spawning area (marked) is out of bounds when fishing for rainbow trout or grayling from the start of October. Details of each angler's daily catch are entered in a log-book, giving number, size and species of fish taken, but unsuccessful anglers do not have to record the fact. NB, In these circumstances, good catch data are available, but fishing effort is underestimated to a varying and unknown degree.

Analysis

The main objective of this exercise is to determine the survival rates of stocked trout, their residence time and changes in stock density. For this we need to calculate, for each type of trout in the fishery:

- The number of fish alive prior to and immediately after each restocking;
- The total mortality and mortality due to causes other than fish capture, each week; and from this we can
- determine the relationship between catch per unit effort and stock (catchability) and learn something of its variability.

The numbers of each batch of trout in the fishery (N_t) at the end of each week during the fishing season can be estimated by the method of Pawson (1982). Leslie analyses (Leslie & Davies 1939) of catch-per-effort successions on cumulative catch following the last restocking each year are used as described by Ricker (1975) to estimate the stock remaining at the end of each season, or at the time of each restocking, on the assumption that losses of trout from the fishery consist of anglers' catches and a constant rate of unreported mortality (*M*). These are then used with the stocking and catch data to determine the changes in trout numbers in the fishery each week, using an iterative process to estimate the coefficient of instantaneous 'natural' mortality *(M)* which would be required to explain the losses from the stock, other than those due to anglers' catches. These might include unrecorded catches, mortality due to predators and/or emigration from the fishery. The use of *M* avoids the successive overestimation of stock levels in the fishery which inevitably occurs when only anglers' declared catches are taken into account, and its value can be determined by the method described by Pawson (1986) and given in Appendix 2.

Unless there are indications to the contrary, the estimated value of *M* can be assumed to remain constant during each fishing season. At a later point in the analysis, it is useful to examine whether there are successively higher estimates of fishing mortality through the year, which would indicate a progressive underestimation of stock numbers (i.e. *M* is over-estimated) and reveal that the calculated numbers of fish available to be caught are unrealistically low given the anglers' recorded catches. However, bias in stock level estimates caused by inaccuracies in the value of *M*, or the assumption that it remains constant and similar for triploid and diploid trout, will have little effect on comparisons of the performance of different trout types in the same fishery (Pawson, 1991).

Stock levels (N_t) at the start of each week of the fishing season can be estimated by successively deducting weekly catches, adding stock where appropriate and, if necessary, making an allowance for unreported mortalities or fish which have otherwise left the fishery (*M*). Once satisfactory estimates of stock levels have been obtained, the coefficient of instantaneous total mortality (*Z*) over any period t is determined as the natural logarithm of the ratio of the stock number at the start and end of each week, N_t/N_{t+1}, from which the instantaneous fishing mortality coefficient *(F)* can be found by subtracting *M*.

$$F_t = ln\,(N_t.N_{t+1}^{-1}) - M$$

The averaged catchability *(q)* of the trout during this period is *F/f*, i.e. the fishing mortality per angler-hour. Since it is unlikely that many anglers would be able to fish selectively for either triploid or diploid trout, or wild brown trout, the same fishing effort *(f)* each week applies to all fish *(F = qf)*. The relative catchability of each trout type is, therefore, given by the ratio of the respective weekly fishing mortalities of each batch and is independent of stock numbers. Values for absolute catchability can be determined if one has accurate fishing effort data and a good estimate of *M*.

6.1.8 Interpretation of results

It is important to recognise at this stage, that interpretation of fishery records, even using the simple model described above, is not necessarily straightforward. Estimates of survival, residence time and population size will depend on the level of catch and release practised in each fishery (which will inevitably vary between anglers, no matter what the fishery rules require) and the subsequent response of the fish. That is, catchability (the relationship between catch rates and population size) cannot be assumed to be constant, nor to be equally affected between triploid and diploid, stocked and wild trout. Experience in conducting this type of experiment contributes considerably to the reliability of the results.

It must also be recognised that the potential impact of stocking with triploids (or any other trout) on wild trout production and population structure may be extremely difficult to detect, given the variability inherent in such populations. The National Principal Brown Trout List (available from the Environment Agency) provides an inventory of survey time-series data of sampled trout stocks, though the actual data are held on a database which is not yet readily accessible. Taken with information on the extent of stocking, obtained from the Section 30 database, it may be possible to make some evaluation of the impact of stocked fish on populations of wild trout. If these data prove to be inadequate for the purpose, targeted sampling will be necessary.

6.2 Task 4 (ii) Project Objective

To design a cost-effective programme of investigations to evaluate the range, nature and degree of impacts of triploid (as compared with diploid) stocking on wild trout populations (by March 2006). Behavioural issues of possible abnormal levels of aggression, cannibalism (including egg eating), and interference with wild breeding, each need to be addressed directly in some way by the project.

6.2.1 Introduction

The Agency's policy on stocking native trout fisheries under the National Trout and Grayling Fisheries Strategy may recognise that, because of genetic or other perceived detrimental effects of using fertile farmed diploid brown trout, only triploid farmed trout will be allowed to be stocked, provided that the potential impact of triploids on wild trout is considered to be acceptable. Phase 2 of this project will, therefore, need to examine whether these impacts are significant at a (wild trout) population level in field situations. This section explains how a risk assessment can be used a) to identify those issues that are most likely to influence the policy (and thus for which information is needed and further research undertaken) and b) provide a robust and defensible mechanism for decision making.

6.2.2 Risk assessment of introducing triploid trout

The problems caused by introductions of non-native species are now recognised as one of the most significant drivers of environmental change world-wide. Indeed, exotic species invasions are considered as second only to direct habitat destruction as a cause for the decline in global biodiversity (Shine et al. 2000). Rivers are amongst the freshwater ecosystems most susceptible to invasion (Ross 1991) and, if this is irreversible, it is arguable whether any intentional introductions are acceptable (Smith et al. 1999). Therefore, identification and assessment of hazard risk are essential for reducing or eliminating the potential impacts of introduced organisms.

This awareness of the impacts associated with species introductions has led to a number of international initiatives (for example, the International Plant Pest Convention of the United Nations (FAO, 1951)), which explicitly require some element of risk assessment prior to the importation of non-native organisms. Historically, these risk assessments have focused on the impacts of disease and pathogenic agents on species of high economic value to agriculture, fisheries, horticulture and forestry. However, implementation of the Convention on Biological Diversity (CBD, 1992) has initiated a shift in focus to encompass the impacts to biodiversity. These initiatives have led Defra to fund a project to develop a risk assessment framework for non-native fishes (Copp, Garthwaite & Gozlan, unpublished, Gozlan, Copp & Garthwait, unpublished). This involves a two-phase protocol: 1) *hazard identification*, which uses the Fish Invasiveness Risk Assessment (FIRA) scoring system to categorise species as high or low risk (rejected or acceptable risk); and 2) for those species classed as high risk by FIRA, *hazard assessment*, which uses a Pest Fish Risk Assessment (PFRA) system to determine quarantine status.

Whilst triploid brown trout are not a non-native species *per se*, they do constitute a form of 'exotic' organism, and thus the biological components of the FIRA scoring system are relevant. These include risks associated with the:

- introduction of non-native species (in this case, diploid or male triploid trout resulting from <100% all-female triploidization) that might eventually establish a reproducing population (or interfere genetically with native trout),
- displacement of wild trout from habitat (through aggressive behaviour)
- increased energetic costs (and resulting reduced growth and reproductive capacity) of wild trout due to territory defence and competition for food
- modification (destruction or deprivation) of habitat or feeding resources used by wild trout
- hosting of native or exotic diseases, and
- ramifications for recruitment success in wild trout, whether it be interference (during spawning) or predation by triploids on wild trout eggs and/or fry.

The overall impacts resulting from the introduction of triploids, and central to the assessment of fish pests (PFRA), include the:

- identification of past environmental impacts where triploids have been introduced (in existing fisheries or other countries, for example),
- estimation of the severity of the environmental impacts in the recipient waters,
- identification of vulnerable species and groups (age classes) in the recipient waters
- estimation of the likelihood of an environmental impact if triploids are introduced,
- availability of suitable habitat for triploids within the recipient area
- estimation of the likelihood of triploids spreading via natural dispersal beyond the intended zone of introduction,
- estimation of the likelihood of human-assisted dispersal beyond the intended zone of introduction,

- estimation of the potential rate of dispersal beyond the intended zone of introduction, and
- feasibility of containment of triploids (once released) and diploids (if present as an unintentional introduction)

An assessment would focus on any difference in these impacts on wild stocks (other than by cross-breeding) between stocked triploid and diploid trout.

In view of the limited information available on triploid trout in the wild, the risk assessment helps to identify potential risks, based on speculation about possible outcomes and, in doing so, raises issues that might otherwise not receive consideration. The assessment should thus be carried out early in Phase 2 in order to identify priority topics for the experimental study. The results should be presented to clearly address the areas of concern raised by stakeholders.

It is recognised that the results of any assessment are only as good as the available information on the constituent elements, and there is a paucity of relevant information for triploid (or even diploid) brown trout. Consequently, these assessments will rely initially on bibliographical information obtained from the literature review carried out under Task 1, to highlight the factors most likely to be associated with potential impacts due to stocking with triploid brown trout and, therefore, those that require further quantification. The biological results (growth, condition, survival) and management evaluations (fishery performance, angler perception) pertaining to the range and nature of potential impacts of triploid stocking on wild brown trout, will be incorporated once they are obtained in the sampling programme designed for Phase 2 (i) of this project (see 6.1). Relevant information on the direct impacts of stocked trout on wild populations is available in Agency R&D Note 490 "The effects of stocked Brown Trout on the survival of wild fish populations", and the Game Conservancy are currently involved in a large scale study of interactions between wild and stocked (diploid) trout and Agency R&D on the impacts of stocked trout on SAC species has just started.

The sampling programme set out under 6.1 above should be designed to provide quantitative input to the risk assessment, though the funding scope for Phase 2 will determine whether the more qualitative information such as behavioural interactions (spawning, competition for habitat and food, predation and migration) between wild and stocked fish need to be modelled or could be measured directly by observation studies. It is likely that the most fruitful source of such information, for example whether triploid trout usurp favoured feeding locations or interfere with spawning of wild trout, is from river keepers and anglers with a scientific background (most anglers are notoriously biased in their opinions with respect to fish behaviour). Their views can be validated, if this is thought worthwhile, by studies using direct observation and acoustic or radio telemetry (for example) in a limited number of fisheries.

Similarly, features such as transmission of fish diseases could be considered to determine whether triploid trout host parasites or pathogens that might be detrimental to wild fish populations. Questions concerning the potential for dispersion of triploid trout beyond the point of introduction, from a consideration of past introductions as well as tagging/telemetry information on migratory patterns/behaviour, might also be addressed in this way. More importantly, for this project, the model can also take into consideration the wider economic, ecosystem and societal issues of introducing triploids into the wild, and provides scope to integrate into the assessment process the anglers' perceptions on the merits or otherwise of extended stocking with triploid trout. Whilst this can be used to judge the potential acceptance of such the Agency's policy, it is likely that decisions to adopt this policy will rest largely on the demonstrable relative level of impacts that triploid (and stocked diploid) trout have on wild brown trout.

The importance of risk assessment is based on the statement that "Significant risk of adverse impacts on wild trout stocks would constitute a basis for refusal of Section 30 consent." It forces the evaluator to consider objectively a range of issues (environmental, societal, economic), though the biological and environmental components of the risk assessment are of particular concern in the present case, and would provide the EA with an objective tool for determining section 30 status. However this is done, it is necessary to rank and compile the real and perceived hazards (using what is known and suspected, including available information about other triploid salmonids in other parts of the world) to determine whether, overall, the introduction of triploid trout possesses acceptable or unacceptable risks to the aquatic environment in general and to wild trout stocks in particular, using the precautionary principle as the underlying philosophy.

6.3 The Elements of the Programme are Summarised Below

6.3.1 Risk assessment

- Carry out hazard identification protocol to determine whether triploid trout represent an acceptable or unacceptable risk to wild stocks of brown trout.
- If triploid trout are deemed to be of high risk, then carry out hazard assessment to determine the limits within which Phase 2 of the project should be undertaken so as to evaluate the extent of the risk(s) under experimental *in situ* conditions.

6.3.2 Choosing a fishery

- Where are triploids stocked, for how long and how many?
- Is there access to the suppliers, to select and mark batches before/at stocking
- Which stocked fisheries that also contain wild brown trout are amenable to the study?
- Do they have stocking and catch records?
- Is there any existing population sampling (Agency wild trout sampling programme, or other)?
- Select 5 suitable fisheries, one each from a chalk stream, a lowland (non-chalk-type) river, a productive upland and an oligotrophic upland river, and a stocked lake with naturally-spawning brown trout in feeder streams (?).

6.3.3 Stocking, catch and population data

- Stock with equal marked batches or "normal"(size distribution) stock trout
- Batch marks or individual tags
- Statistically sufficient numbers required - what duration of trial is required at each fishery to give meaningful results?
- All anglers using the fishery must enter daily catch data, including fish measurements
- Protocols for trials, tailored to fishery, but with same features - explanation and publicity
- Angler perception via fishery questionnaire or interview (note government constraints on surveys)
- Behavioural study using tagged (visual/telemetric) fish (depends on residence time)
- Population sampling by electrofishing (focussed on wild fish)

6.3.4 Analysis

- Methods to estimate survival and residence time covered by references and outlined in boxes and Appendix 2.
- Growth by increment in length, stocking to recapture, and/or scale sample back-calculation
- Behavioural data will need non-parametric comparisons of impact.

6.3.5 Illustrative schedule

Autumn/Winter 2003: Contact fisheries and farms, arrange for marked batches to be stocked for the 2004 season, and discuss recording protocol with anglers in 2 fisheries; carry out hazard identification.

Spring/Summer 2004: Monitor catches in 2 selected fisheries and those adjacent (for strays); arrange for behavioural observations (plus telemetry - Home Office Approval) on residual or newly introduced triploids and diploids, including setting up recording system; electro-fishing for samples, if necessary.

Autumn/Winter 2004: Observe stocked fish interactions with wild trout through spawning, including sampling of triploids and diploids to look for evidence of egg or fry feeding (late winter/early spring). Prepare analysis of catch/growth etc data and arrange for marked batch releases in 2/3 other fisheries, plus further releases in 2 original fisheries (experience from first year will facilitate this).

Spring/Summer 2005: Monitor catches in all 4/5 fisheries and those adjacent (for strays); electro-fish for population samples; arrange for further behavioural observations on triploids and diploids , depending on previous year's results.

Autumn/Winter 2005: Observe stocked fish interactions with wild trout through spawning, including sampling of triploids and diploids to look for evidence of egg or fry feeding. Prepare analysis of catch/growth etc data and results behavioural study. Finalise risk analysis, taking advantage of information provided in other studies of stocked trout impacts on wild fish.

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APPENDIX 1
BIBLIOGRAPHICAL INVENTORY ON TRIPLOID TROUT

This inventory gives the full reference list (67) on ASFA, Scirus and Science direct, using keywords salmo*, tetraploid, ploidy, pressure shock, heat shock, gynogen*, cytochalasin, trout, and a few names such as Benfey. Several references found were not used in the review as they were of little relevance. This is followed by annotated key references to triploidy in brown trout.

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Annotated key references to triploidy in brown trout

Altimiras, J., Axelsson, M., Claireaux, G., Lefrançois, C., Mercier C., and Farrell, A. P. (2002). Cardio-respiratory status of triploid brown trout during swimming at two acclimation temperatures. *Journal of Fish Biology,* **60(1)**, 102-116.

> At 14 degree C, standard metabolic rate (75.1 mg O sub(2)h super(-1)kg super(-1)), routine metabolic rate (108.8 mg O sub(2)h super(-1)kg super(-1)), active metabolic rate (c. 380 mg O sub(2)h super(-1)kg super(-1)), critical swimming speed (U sub(crit)1.7 BL s super(-1)), heart rate 47 min super(-1)), dorsal aortic pressure (3.2 kPa) and ventilation frequency (63 min super(-1)) for triploid brown trout *Salmo trutta* were within the ranges reported for diploid brown trout and

other salmonids at the same temperature. During prolonged swimming (c. 80%U sub(crit)), cardiac output increased by 2.3-fold due to increases in heart rate (1.8-fold) and stroke volume (1.2-fold). At 18 degree C, although standard and routine metabolic rates, as well as resting heart rate and ventilation frequency increased significantly, active metabolic rate and certain cardio-respiratory variables during exercise did not differ from those values for fish acclimated to 14 degree C. As a result, factorial metabolic scope was reduced (2.93-fold at 18 degree C v. 5.13-fold at 14 degree C). Therefore, it is concluded that cardio-respiratory performance in triploid brown trout was not unusual at 18 degree C, but that reduced factorial metabolic scope may be a contributing factor to the mortality observed in triploid brown trout at temperatures near 18 degree C.

Arai, K. and Wilkins, N.P. (1987). Triploidization of brown trout (*Salmo trutta*) by heat shocks. *Aquaculture*, **64(2)**, 97-103.

This study was made to optimise heat shock conditions for producing triploidy in the brown trout, *Salmo trutta* . Heat shock at 29 degree C for 10 min duration, initiated between 5 and 45 min after insemination, gave high frequencies of triploid embryos (77-91%) as assessed by chromosome observation. Shocks initiated between 90 and 260 min following insemination had no effect on polyploidization. Other groups heat shocked at 29 degree C for 5-15 min duration initiated 10 min after insemination resulted in moderate rates of triploidy (50-63%). A high temperature shock of 32 degree C for 6 min duration gave 100% triploidy but a lower temperature shock of 26 degree C, even for 30 min duration, had only a moderate effect (57%). Rates of hatching were generally decreased in the groups giving moderate to high frequencies of triploidy. In most treated lots haploid embryos were observed and were considered to be a cause of decreased survival.

Benfey, T.J. (1999). The physiology and behaviour of triploid fishes. *Reviews in Fisheries Science* **7(1)**, 39-67.

Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. Artificially produced triploids generally differ from conspecific diploids in three fundamental ways: they are more heterozygous, they have larger but fewer cells in most tissues and organs, and their gonadal development is disrupted to some extent. Despite these basic biological differences, triploids are similar in most respects to diploids when examined at the whole animal level. The only clear differences relate to the effects of impaired gametogenesis on the reproductive physiology and behaviour of triploids, especially in females. Other apparent differences include reduced aggressiveness, occasional specific morphological abnormalities, and inferior performance when reared under suboptimal conditions. The causes of these latter two problems are poorly understood but must be addressed if triploids are to be used more extensively.

Brydges, K. and Benfey, T.J. (1991). Triploid brown trout (*Salmo trutta*) Produced by hydrostatic pressure shock. *Bull. Aquacul. Assoc. Canada* **91(3)**, 31-33.

The goal of this study was to determine the optimum pressure treatments for the induction of triploidy in brown trout (*Salmo trutta*). Three variables were examined: duration of the pressure shock, magnitude of the pressure shock, and time after fertilization for start of the pressure shock. High yields of triploids were obtained from all treatments except those which were started within 15 minutes of fertilization. The optimum treatment found was in the range of 5.5 to 6.5 min at 9500 to 10500 psi, applied 25 to 30 min after fertilization at 8.5 degree C.

Lincoln, R. (1996). Progress towards the commercial production of triploid brown trout. *Trout News* **22**, 23-28.

Concern over maintaining the genetic integrity of wild populations of brown trout has highlighted the desirability, in certain situations, for producing sterile fish for restocking. The most effective method currently available for large scale sterilisation of trout is by the induction of triploidy. The purpose of the article is to draw the attention of fish farmers to information available on the induction, characteristics and uses of triploid brown trout.

Mercier, C., Axelsson, M., Imbert, N., Claireaux, G., Lefrançois, C., Altimiras J. and Farrell, A. P. (2002). *In vitro* cardiac performance in triploid brown trout at two acclimation temperatures, *Journal of Fish Biology,* **60(1)**, 117-133.

The maximum values for heart rate (f sub(H)), stroke volume (V sub(H)), cardiac output (Q) and myocardial power output, measured in vitro with a perfused heart preparation, as well as the isometric force-frequency relationship for atrial and ventricular muscle strips, in triploid brown trout *Salmo trutta* were all comparable with established information for diploid rainbow trout *Oncorhynchus mykiss.* Therefore, it was concluded that triploidy is not associated with a major deficiency in maximum cardiac performance. However, a heightened sensitivity to ryanodine was discovered, which indicated an enhanced role for the sarcoplasmic reticulum in excitation-contraction coupling in these triploid fish. It is suspected that the enhanced role of the ryanodine receptor may be a cellular compensation related to larger cardiac myocytes. It was also clearly established that there was a plateau in maximum cardiac performance between 14 and 18 degree C and this plateau might be a contributing factor to the reduced factorial aerobic scope and increased fish mortality observed at 18 degree C.

Moffett, I.J.J. and Crozier, W.W. (1995). An investigation into the reproducibility of triploid brown trout, *Salmo trutta L.*, production using heat shock. *Aquaculture Research* **26(1)**, 67-70.

A single heat shock of 28 degree C for 10 min, applied 15 min after water activation to five different batches of brown trout, *Salmo trutta* L., ova and

replicated within one batch produced triploid rates ranging from 22% to 100% and variable survival to hatch ranging from 9.4% to 47.9%. Variability was greater among batches than among replicates. In general, the treatment reduced survival to hatch by 50%. The range of triploid yields obtained from a single heat shock regime is discussed in terms of potential advantages to aquaculture.

Myers, J.M., Powell, S.F. and McAndrew, B.J. (1995). Induction of tetraploidy in brown trout, *Salmo trutta* L., using hydrostatic pressure. *Aquaculture Research* **26(3)**, 229-232.

Sterile aquatic organisms may be potentially beneficial in aquaculture and fisheries management. Attempts to produce sterile animals en masse using hormonal and/or genetic techniques have varied in their success. Public concern regarding the consumption of hormonally treated fish has limited the use of steroids to retard or alter sexual development in production fish. Additionally, hormone treatments may have detrimental effects on growth and smoltification. Genetic methods of sterilization may be preferable to hormonal treatments. Triploid fish appear to be functionally sterile, although male triploids to develop secondary sexual characteristics. Brown trout, *Salmo trutta* L., is an important species both to sport fisheries and, to a lesser extent, to aquaculture. Triploidized individuals would be useful in preventing maturation and eliminating the risk of stocked fish genetically contaminating native strains. The production of tetraploid in brown trout could improve the production efficiency and growth of subsequent triploid brown or tiger trout. In the absence of previous studies, this pilot project was initiated to assess the feasibility of inducing of tetraploidy in brown trout.

Quillet, E., Foisil, L., Chevassus, B., Chourrout, D. and Liu, F.G. (1991). Production of all-triploid and all-female brown trout for aquaculture. *Aquatic living resources/Ressources vivantes aquatiques. Nantes*. **4**(1), 27-32.

Heat shocks effective for the production of triploid brown trout (*Salmo trutta*) were optimized: 28 degree C shocks lasting 10 to 15 minutes and applied 5 minutes after fertilization induce very high rates of triploidy (close to 100%) without causing much mortality; they can therefore be proposed for mass production of triploid brown trout. Female homogamety in that species is also demonstrated from analysis of gynogenetic progenies and progenies of sex-inverted females. Although the efficiency of masculinizing hormonal treatments requires further improvement, production of all-female sterile populations is now possible in this species promising for European sea-farming.

Solomon, D.J. (2003). The potential for restocking using all-female triploid brown trout to avoid genetic impact upon native stocks. *Trout News* **35**, 28-31.

All-female triploids would appear to satisfy the requirements for a fish for stocking that have all the beneficial attributes of diploids without the genetic risk to the native stock. They appear to be in extensive use in the south of England at

least, being preferred for their higher over-winter survival in both farm and river. Consideration of a firm policy for their use in situations where a genetic risk is perceived with the use of diploids would appear to be a sound and justified development. Before that is done, however, it would be prudent to examine in more detail the performance of stocked all-female triploids, especially with respect to possible interactions with wild fish.

APPENDIX 2
THE ANALYSIS OF FISHERY PERFORMANCE, FROM BASIC PRINCIPLES

The number of fish alive at the beginning and end of each stocking period

Leslie's method of estimating population abundance from catch and effort successions (Leslie & Davis, 1939) can be used as described by Ricker (1975) according to the formulae:

$$\frac{C_t}{f_t} = qP_t \qquad (1)$$

where C_t is catch during time interval *t;* P_t is the mean population surviving during *t;* f_t is the fishing effort during *t* angler hours or rod days; and *q* is the fraction of the population taken by one unit of effort, i.e. 'catchability'.

That is: catch per unit effort is related to stock size by the fishes' catchability, and

$$P_t = N_o - K_t \qquad (2)$$

where N_o is the original population size and K_t is the cumulative catch up to the start of period *t,* plus half the catch during *t.*

Combining (1) and (2)

$$\frac{C_t}{f_t} = qN_0 - qK_t,$$

and if C_t/f_t is plotted against K_t it should give a straight line with a slope of *q* and an intercept on the $x(K_t)$ axis at N_o, assuming that there is no `natural' mortality (i.e. all fish deaths are due to fishing and are recorded in C_t) and *q is* constant.

It should be noted that if there are any fish deaths not reported in C_t, these *q* values will be overestimates. Similarly, it is to be expected that N_o's are underestimated, although the estimates of the number of fish at the end of the period (N_o - C_t) may be more accurate in absolute terms, providing *q* remains relatively constant.

It is likely that there will be a greater correlation between stock and catch per unit effort for all trout combined than for either triploids, diploids or wild fish separately, since it is unlikely that anglers will discriminate between them during fishing.

Determination of mortality rates

The rate of change in numbers (*N*) in a population can be expressed by

$$\frac{dN}{dt} = -ZN$$

where Z is the instantaneous total mortality coefficient. The number alive at time t (N_t) is given by:

$$N_t = N_0 e^{-Zt}$$

and

$$Zt = \ln \frac{N_0}{N_t}.$$

An estimate of Z during each inter-stocking period can be made from the values of N_o and N_t obtained above. Since total mortality is made up of deaths due to fishing and to other causes,

$Z = F+M$

where F is the instantaneous coefficient of fishing mortality and M is the instantaneous coefficient of `natural' mortality. The relationship between catch, stock and mortality is given by

$$C_t = N_0 \frac{F}{Z} (1 - e^{Zt})$$

from which F can be estimated, using an assumed value of M (initially = 0, say), which can be tested if necessary by recalculating N_o, Z and F and continuing the iteration until a "best" estimate of per week is found. If necessary, estimates of the number of fish remaining at the end of each inter-stocking period, plus the numbers stocked less those caught in the next period, can be compared with stock numbers estimated using Leslie's method to indicates the likely level of mortality other than that due to fishing. However, the effects of wrongly estimating M are not important in comparisons between stocked fish caught within one fishing season, and a value of M = 0 can probably be accepted for most analytical purposes

Testing the relationship between catch, effort and stock

In order to investigate the variability in q between batches of fish, the following model can be used. The basic data required for the model are the numbers of stocked fish, the weekly catches, and estimates of fishing effort, initial population size (N_o) and M. Although F and M are instantaneous coefficients of mortality and act simultaneously, sufficiently accurate results for our purpose can be obtained using 1 week as the basic unit of time. An estimate of N_t can be made by calculating the numbers of fish remaining after 'natural' mortality each week, and then subtracting the number of fish caught during that week. Thus:

$$q = \frac{\ln \frac{N_t}{N_{t+1}} - M}{f_t}$$

This method of calculating q takes into account the changes in stock levels due to fish being caught and to their removal from the fishery by other means (predation, unreported catches, etc.) during each period. The relationship between instantaneous values of q and the number of trout in the fishery at any time is proportional to the catch-per-unit of fishing effort (C_t/f_t).

The least well quantified variable in these calculations is the absolute level of stock at any one time which, if in error, would introduce a bias to q values ($q = C_t/f_t/N_t$). The effect would be largest at restocking times and, if necessary, the validity of stock estimates can tested by determining q values for each of several days before and after each restocking. The observed variations in q at these times also enable us to examine the response of the trout population in the fishery to the introduction of new fish, in so far as the fishes' susceptibility to angling is concerned. It should be noted that though the value of M particularly influences the estimates of q at low stock levels, trends in q are less affected than the absolute levels.