A preliminary survey of Cantley Marsh, Gillingham Marsh and Sculthorpe Moor (Norfolk) to determine the feasibility of translocation and re-introduction of *Tolypella intricata*

First Report to the Norfolk Biodiversity Partnership

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Contents Page

Abstract

Tolypella intricata is listed as a 'vulnerable' Red Data Book charophyte species within Britain. Over the latter half of the 20th century its distribution reduced from 42 recorded sites pre 1970, to 4 sites in 1992. A Biodiversity Action Plan (BAP) exists for the species which includes measures to re-establish the species at historical sites within Norfolk. A new record of the species was made at Sculthorpe Moor Norfolk in 2006. To contribute to the implementation of the BAP at a local level, the site conditions and population were assessed by physical survey and water quality analysis. Results were compared with similar surveys of two further sites in Norfolk UK (Cantley and Gillingham Marshes) where the species had previously been recorded (Cantley and Gillingham Marshes) to assess the possibility of re-establishment of the species at these sites. The survey results were viewed in the light of recent PhD research which investigated water quality criteria required for successful establishment of British charophytes generally. The results of the study indicated that it would be improbable to achieve successful re-establishment of the species at either Cantley or Gillingham Marshes due to unfavorable winter water quality, particularly dissolved nitrate derived nitrogen concentrations (> 0.5 mgl^{-1}). However there was considerable scope for increasing the 'within site' distribution of the species at Sculthorpe Moor, where water quality was recorded within recommended limits.

1. Introduction

1.1 *Tolypella intricata*

Tolypella intricata is a rare Charophyte listed in the Red data Book of Britain and Ireland and classified as 'Vulnerable' (Stewart 1992). The most recent published information (UK BAP Plans 2005) states that it has been found at six British sites since 1970: ponds in Inglestone Common, Gloucestershire (seven colonies), one site in Cambridgeshire (four colonies), and one site in each of Suffolk, Norfolk, Somerset and Worcestershire. The species reappeared at the Somerset site following ditch clearance in 1989, but has not been seen since. It was once more widespread, being recorded from 42 localities pre-1970, most of which were in southern and eastern England, but it also extended as far north as Durham. In 2006 it was discovered in a ditch at Sculthorpe Moor (Norfolk) by a local naturalist Geoff Nobes. It is scattered throughout Europe extending to southern Scandinavia, the Black Sea and North Africa, but is rare in the Mediterranean area. In the UK it receives general protection under the Wildlife and Countryside Act 1981.

1.2 *Habitat*

The species is described as an inhabitant of alkaline water in pools, canals, ditches, poached edges of ponds and wheel-ruts that are dry during the summer months. It is often a winter or spring annual, able to withstand ice-cover and producing ripe spores as early as April or May. Plants then often disappear by early July. It is not very competitive and benefits from disturbance which keeps down other vegetation. Thus in ditches, it often reappears after clearance work, and cattle disturbance, which clears marginal vegetation and exposes sediments around pools, is often beneficial, (Stewart, 1992). Although commonly a small plant (<20cm), (Stewart, N. pers com) in the Netherlands it has been observed growing in shallow lakes at heights of up to 60 cm (Pankhurst, T. pers com).

1.3 *Current factors assumed to be causing loss or decline*

There are two common factors to which much of the decline in distribution of this species have been attributed, (1) a lack of disturbance (often due to a reduction in grazing and ditch clearance) leading to displacement of this species by more competitive vegetation, and (2) falling water-tables due to over abstraction for intensive arable farming industry and urban use. The extent of these problems needs further investigation (www.BAP 2005).

1.4 *Suggested conservation measures*

Unless there are other factors controlling the vegetation, ditch and pool sites need to be dug out periodically ideally at least every ten years. As a last resort, spores, or mud containing spores could be collected and transplanted to other suitable habitats in the vicinity. Such action should be undertaken only after adequate trials have been carried out. The translocation should be carefully recorded and results monitored (Stewart 1992).

1.5 *Recent research*.

A recent PhD thesis carried out at the University of East Anglia studied 144 water-bodies at 47 historically noted charophyte sites. The results indicated that raised dissolved nitrate derived nitrogen and heavy some metals (in particular copper and cobalt) may well play a more important role in reducing the probability of charophytes establishing and persisting at a site than the commonly monitored and widely recognized element phosphorus. The limitation mechanisms are by the encouragement of nitrogenous macrophytic competition in the case of Nitrogen, and direct toxicity at very low environmental concentrations (< 50μgl^{-1}) in the case of the heavy metals. Approximate critical thresholds are shown (Table 1).

Table 1. Dissolved element concentration predicted to reduce the probability of charophyte presence at a site to <50% (Lambert 2007).

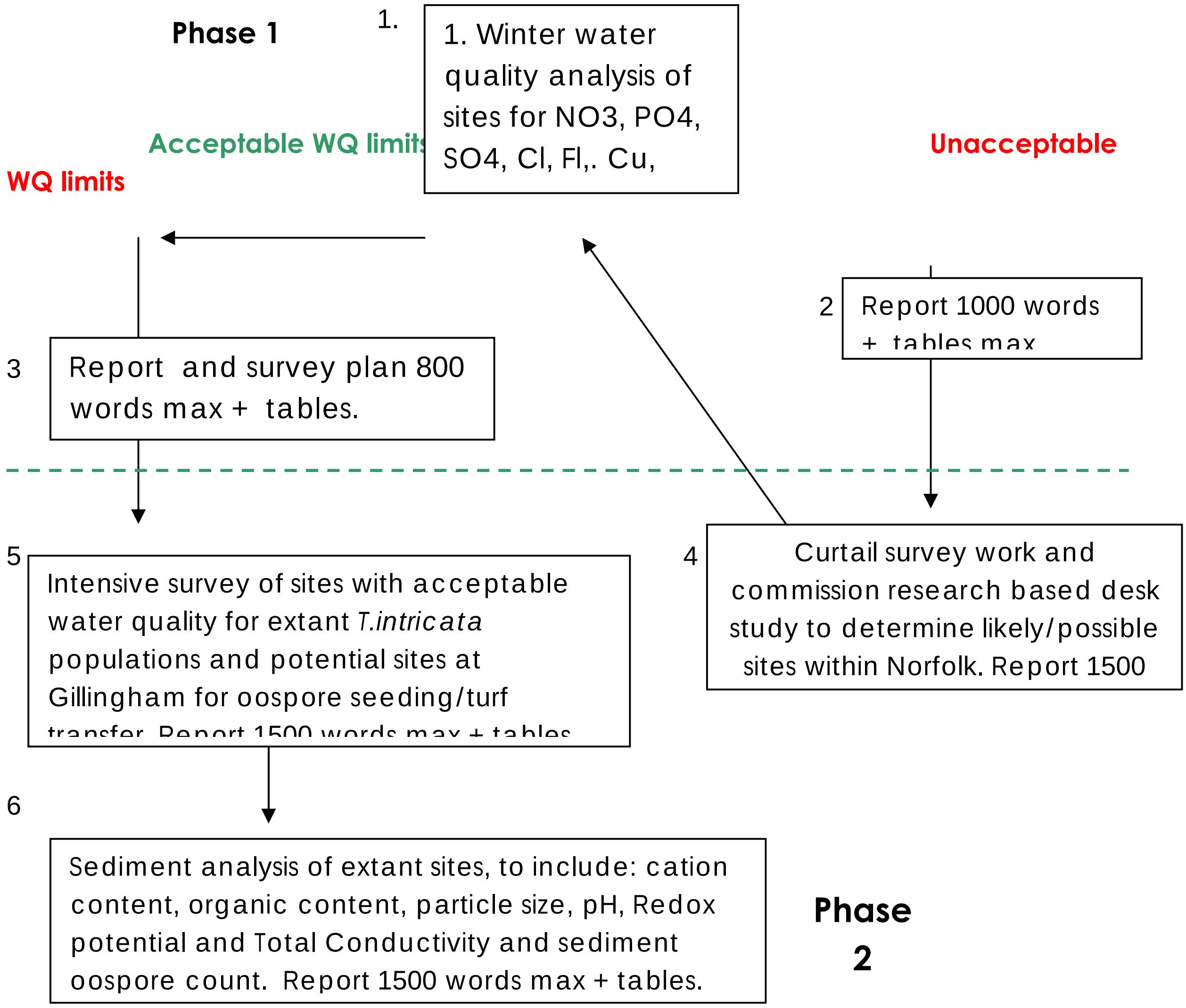
Element	Upper limiting environmental concentration
Nitrate – N	0.5 mgl^{-1}
Phosphate – P	0.020 mgl^{-1}
Cobalt	0.05 μgl^{-1}
Copper	100 μgl^{-1}
Manganese	16 μgl^{-1}

1.6 *Project outline*

An extensive population of *Tolypella intricata* was discovered extending over nearly 50 m of a 1 m wide ditch at Sculthorpe Moor in 2006. In pursuance of recommendations for conservation of the species as put forward by Stewart and Church in 1992, and also in line with National BAP targets (BAP 2005), (Appendix 2), a proposal was put forward to the Norfolk Biodiversity forum to examine the feasibility of using oospores and small turfs from this population to re-introduce the species to two sites where it had previously been recorded in Norfolk, i.e. Cantley Marsh (N. 52.58438, E.1.49561), and Gillingham Marsh (N.52.46781, E. 1.55229). Sites were assessed by comparison of their winter water quality with that determined (Lambert 2007) as likely to reduce the probability of charophyte establishment and persistence.

The project was designed in three phases in order to take account of the outcome of all possible result scenarios. Nine possible action paths were identified (Fig 1): this report summarizes the results of phase one of the project as outlined in the project action paths.

This report details progress and results from phase 1 of the investigations as outlined in the project structure (Fig 1)



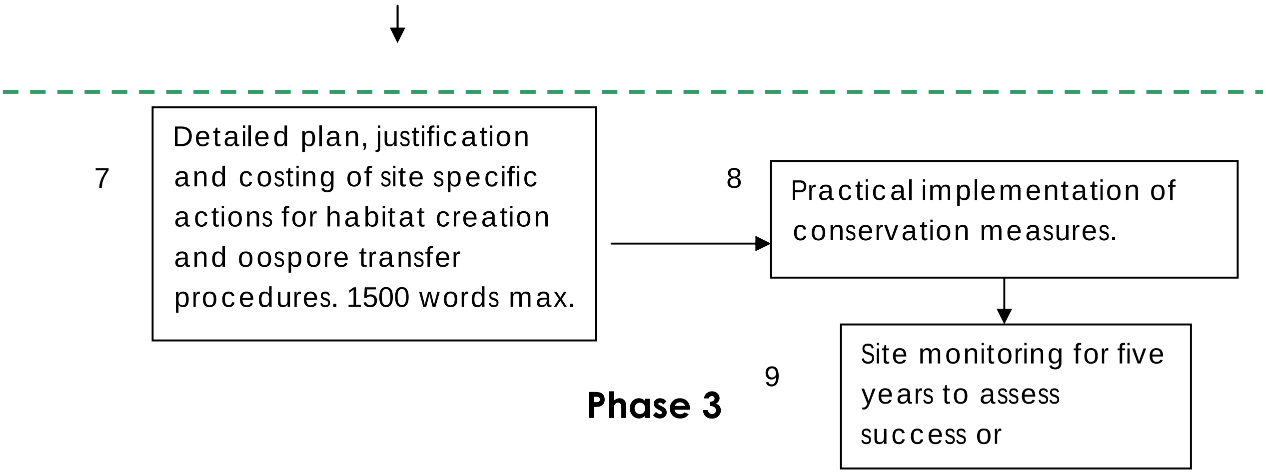


Fig. 1 Action paths for various project results

1.7 *Phase 1 aims*

1.To assess the current site condition of Cantley Marsh, Gillingham Marsh and Sculthorpe Moor with particular reference to water quality and the relative abundance of aquatic macrophytes.

2. To review the results with regard to general conditions that might sustain *Tolypella intricata.*

3. To assess the sites in comparison to conditions that recent research (Lambert, 2007) has determined as a critical limits for the general probability of charophyte presence at a site.

1.7.1 *Specific objectives*

1. To survey 10 sample points at each of the three sites at during February 2008 to determine macrophyte abundance and to collect water samples.
2. To analyze the water samples for a range of water quality determinands, in particular dissolved nitrate, total filterable phosphorus, copper and cobalt and manganese, all of which have been shown to be associated with reduced probability of charophyte survival above critical thresholds in the field (Lambert 2007)
3. To compare the winter water chemistry quality with limiting criteria determined by recent research.

4. To assess the likelihood of success of the establishment of self sustaining populations of *Tolypella intricata* at Cantley and / or Gillingham Marshes by oospores sourced from the oospore bank at Sculthorpe Moor.

2. Methods.

2.1 *Surveys*

2.1.1 *February 2008*

Each site was visited in the last week of February 2008. Sculthorpe Moor and Gillingham Marshes were surveyed for one day each; two days were allocated to Cantley Marshes. The ditches, scrapes and pools at each site were visually examined. Percentage cover of macrophyte within a 1 m quadrat was measured at 10 sample points, chosen subjectively by the author to reflect the apparent range of macrophyte diversity and water quality conditions. At each sample point a 25 ml water sample was taken by syringe filtration via a 2.5 mm Teflon tube through a 4 µm Sartorius filter. The samples were ejected into in 25 ml scintillation vials previously washed in 1 M acetic acid and rinsed with deionized 'milli Q' water and stored onsite in a Coolmatic ™ cool box at 4^0C. Sample points at each site are shown (Appendix 1).

2.1.2 *June 2008*

Sculthorpe Moor and Cantley Marsh were visited again in June of 2008 to observe summer vegetation. This was not in the original scope of the project, but was carried out by the author to compare expected summer aquatic macrophyte communities with actual summer macrophyte communities.

2.2 *Water quality analysis*

All water samples were drawn through a 0.4 μm Satorious™ inorganic membrane filter prior to analysis.

2.2.1 *Dissolved anions*

A Dionex DX100™ packed column HPLC was calibrated for detection of Cl^-, Fl^-, NO_3^-, PO_4^{---} and SO_4^{--} using Dionex™, standard reagents of 100mgl^{-1} and Milli Q water. Limits of detection were extrapolated via linear coefficients at Cl^-, 28μgl^{-1} r^2, 0.995, Fl^-, 14 μgl^{-1}

r^2, 0.997, NO_3^-, 26 μgl^{-1} (5.9 μgl^{-1} N) r^2 0.994, PO_4, 17.8μgl^{-1} (5.8 μgl^{-1} P) r^2, 0.993, SO_4^{---} , 27 μgl^{-1} (9 μgl^{-1} S), r^2, 0.991. Quantitative analysis for the ions was carried out by packed column HPLC (Dionex100™) using 0.2M Dionex ™ bicarbonate elluent at a flow rate of 90 μl min^{-1}.

2.2.2 *Dissolved elements*

Determination for elemental Al, Bo, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P and Zn was by Inductively Coupled Plasma Atomic Emission Spectroscopy (Plasma phase Varian Vista-Pro ICP-AES) (ICP). 1ml of each sample diluted 1:10 with milli Q water and subsequently acidified for ion dissociation at 20% i.e. 0.2 ml 5M HNO_3, + 0.8ml sample. Limits of detection for the standards were commonly extrapolated to 1 ppb.

3. Results

3.1 *Winter surveys (February 2008)*

Charophytes were recorded at Gillingham Marsh and Sculthorpe Moor. *Tolypella intricata* was only confirmed at two survey points, both at Sculthorpe Moor (Appendix 1, Fig 6). Seven further species including the Red Data Book listed *Chara curta* were also recorded *at* Sculthorpe Moor*.* Two species, *Chara vulgaris* and *Chara hispida* were recorded at Gillingham Marsh (Appendix 1, Fig 8). No charophytes were recorded at Cantley Marsh in February (Appendix 1, Fig 7); however, an extensive program of mechanical ditch clearance had recently been undertaken at this site.

Critical concentrations of elements suggested as being limiting for charophyte communities (Lambert 2007) are presented (Table 1). The survey results show that that mean nitrogen concentrations from the ten sample points at both Cantley and Gillingham marshes were generally above the critical threshold (0.5 mgl^{-1}) calculated to be limiting for charophyte persistence (Lambert 2007). Concentrations of cobalt were above those predicted to be limiting (0.05 μgl^{-1}) (Lambert 2007), at Gillingham Marsh (Table 2). The logistic probability (I.e. that based upon recorded presence or absence of charophytes) of a charophytes species surviving at any of the sites surveyed is shown (Fig 3).

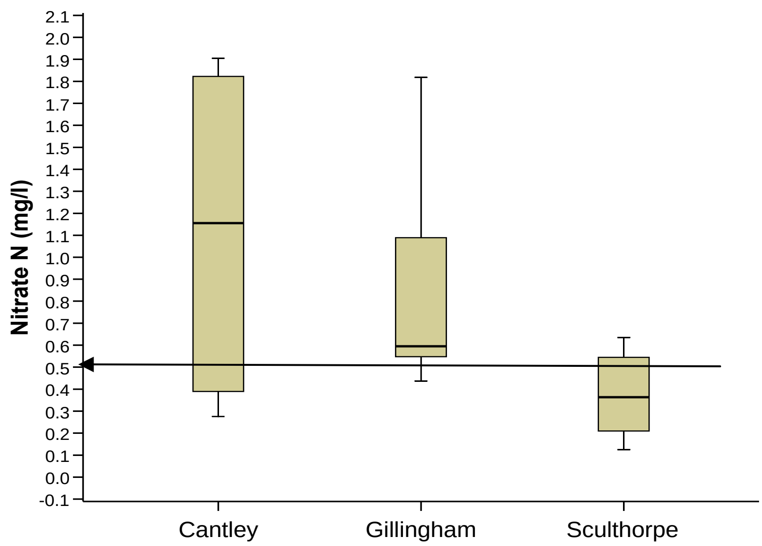
Table 2. Mean concentrations of possible limiting elements (Lambert 2007) recorded at each site in February 2008.

Site		Nitrate-N	PO_4-P	Mn	Co	Cu
Cantley	Mean	**1.86 (↑)**	**	6.45	0.40	12.89
	N	10	10	10	10	10
	Std. Deviation	1.61	0.00	3.03	1.08	3.39
Gillingham	Mean	**0.63 (↑)**	0.01	3.74	**0.56 (↑)**	9.19
	N	10	10	10	10	10
	Std. Deviation	0.40	0.02	.17	0.03	2.52
Sculthorpe	Mean	0.26	**	6.14	0.27	11.30
	N	10	10	10	10	10
	Std. Deviation	0.19	0.00	3.19	.99	5.11

* N, PO_4, Cl, SO_4 (mgl^{-1}); Cd, Co, Cu, Mn (μgl^{-1}).

** = Below limits of detection by HPLC (< 0.02 mgl^{-1})

(↑) Above predicted threshold for charophyte presence derived by logistic regression analysis of 47 historical charophyte sites (Lambert 2007).



Recorded charophyte species	**0**	**2**	**8**

Fig 2. Box plots showing number of charophyte species recorded at each site and median Nitrate derived nitrogen per site.

* The arrow indicates approximate concentration of nitrate derived nitrogen beneath which the probability of a site sustaining charophytes < 5% based on presence or absence data from 47 UK sites. (Lambert, 2007). Bars = median, boxes = 75% inter-quartile range, tails = 95 % confidence limits of the distribution.

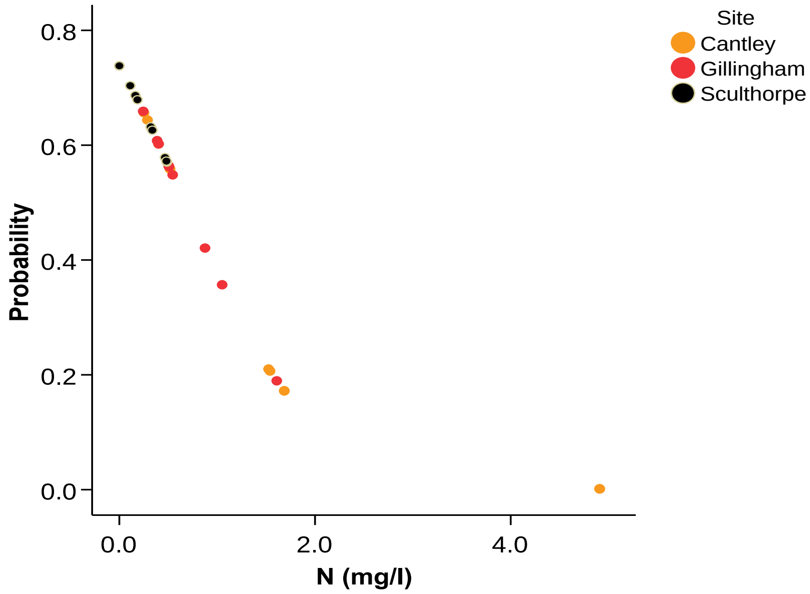


Fig 3. The logistic probability of charophytes being present at the sites based upon recorded nitrate derived nitrogen concentrations in February 2008

3.2 *Walking surveys of sites*

3.2.1 *Cantley marshes (June 2008)*

The overall impression of the ditches was of a eutrophic flood relief system set on alluvial sediments. Water quality was generally poor with apparently high suspended solids leading to low water clarity. Macrophytic angiosperms were abundant relatively species rich, however ditches were often dominated by beds of floating filamentous algae (mainly *Cladophora spp*). Floating species such as *Hydrocharis morsus ranae and Lemna spp.* were dominant in many ditches (Fig. 4 a). The location of the recording of *Tolypella intricata* in 1999 was dominated by marginal floating *Cladophora spp* (Fig 4 b).Occasionally in isolated locations, cut off from the main drainage system, clear water was apparent, indicating water of lower nutrient status, possibly of spring fed

origin. In two such ditches, an abundant growth of *Chara vulgaris* was recorded (Fig 4.c).

a **b**

Fig. 4. Photographic records of a walking survey of Cantley marshes June 2008 (a). *Lemna spp* and *Hyrocharis sp*. dominated ditch, (b). Filamentous algal bloom (*Cladophora* spp.) at location of the most recent record (Martin .G, 1999) of *T. intricata* at Cantley marshes; (c) ditch containing abundant growth of *Chara vulgaris.*

3.2.1 Gillingham (Feb 2008)

The drainage ditches at Gillingham marshes were found to be in varied condition, those to the west of the Gillingham road being in an advanced state of succession, (Fig 4, a & b). Those on the eastern side of the road were clearer and contained abundant growth of *Chara vulgaris* and *Chara hispida* (Appendix 1 Fig 8)

Fig 5. Images from Gillingham Marshes (Feb 2008). (a) succeeded drainage ditch, (b) more open ditch, (c) *Chara vulgaris* coated in filamentous algae in perimeter ditch of western field, (d) bucket of cattle vitamin supplement leeching into a dried ditch (e) contents of cattle supplement.

Chara vulgaris was recorded in the northern and eastern perimeter ditch (Appendix 1, Fig 8), however the plants were emergent from dense beds of filamentous algae (mainly Spirogyra). The succeeded ditches on the western side of the Gillingham road were also littered (Fig 5d) and in need of clearance. The fields were obviously grazed, although not at the time of survey. In one ditch a 10L bucket of cattle 'lick' supplement was abandoned and being allowed to leech into the water-course. Contents of the dietary supplement are clearly marked on the label showing metal concentrations of copper at 1000 $mgkg^{-1}$, cobalt, 80 $mgkg^{-1}$and manganese, 1200 $mgkg^{-1.}$.

3.2.2 ***Sculthorpe Moor (June 2008)***

The ditch at Sculthorpe Moor where *T. intricata* was first recorded by Geoff Nobes in 2006 (Fig.6.a & b) was completely succeeded by grasses in June 2008, (Fig.5.c.). This was apparently due to a lack of grazing on the site. Changes in water level management during 2008 had also resulted in the drying out of the newly cut ride where six species of charophyte had been recorded in February 2008 (Fig 5.d).

Fig 6. (a) Ditch at Sculthorpe moor where *T. intricata* was recorded in 2007, (b) desiccating remains of fertile *T. intricata* in the ditch in August 2007. (c) Condition of the ditch in June 2008, (d) drained ride at Sculthorpe moor (August 2008) where 6 species of charophyte were recorded in February 2008 immediately after scrub clearance.

4. Discussion.

The results of the surveys show:

1. The highest number of charophyte species (8) was at Sculthorpe Moor in February 2008. The location at this site which held the greatest species richness of charophytes was a freshly cut 'ride' at a location 300m W of the original *T. intricata*

ditch. A new population of *T. intricata* was discovered growing in a freshly cut drainage ditch close to the site reception centre. These findings indicate that with physical intervention and water level management, the site holds potential for managed development of it charophyte community.

2. The richness of recorded charophyte species at the three sites declined significantly with winter mean Nitrogen concentrations on the days sampled, this trend follows recent research findings indicating that that Nitrogen is a key element for limiting the diversity of submerged macrophyte communities generally at >2 mgl^{-1} nitrate – N (James *et al.*, 2005), and charophyte survival specifically at >0.5 mgl^{-1} nitrate –N (Lambert 2007).

3. Phosphorus derived from phosphate was only detected at above levels of detection (0.02 mgl^{-1}) at three sample points and was not considered to be a threat to charophyte existence at any of the sites surveyed.

4. At Gillingham Marshes two sample points heavily weighted the mean cobalt concentration to above the predicted limiting threshold for cobalt. Generally the heavy metals cobalt and copper, and the transition metal manganese were not generally recorded at concentrations likely to limit charophytes according to limits quoted (Lambert 2007).* It might be hypothesized that the cobalt 'spikes' in water quality may have been associated with leeching from a submerged cattle lick. The link between cattle feed supplements and localized heavy metal water pollution would benefit from further research.

*** This was a very limited water sampling effort, water samples were taken on only one visit, hence there is no reflection of temporal variation in the sampling regime.**

The increased number of charophyte species (8) and generally lower recorded February nitrogen concentrations (<0.5 mgl^{-1}) at Sculthorpe Moor would suggest that this site has potential for further expansion of current ditches and rides to increase the 'within site' distribution of the species.

Speculatively, the recording of *Chara hispida* and *Chara vulgaris* at both Cantley (June 2008) and Gillingham marshes (Feb 2008) suggests that these two robust species might be better adapted to cope with or benefit from raised nitrogen concentrations and this subject merits further research.

The increased nitrogen concentrations recorded at Cantley Marsh are most probably linked to two causes, (1) winter flooding from the R. Yare, and (2) the large overwintering flock of migrant geese (reportedly > 7000, RSPB Warden, pers com) that have established themselves over recent years.

During the February surveys large amounts of goose excrement was noted along the margins of ditches, most of which must be counted as a net import of nutrient load to the site via the geese from daytime grazing on the adjacent sugar beet fields. There is now a substantial body of evidence to show that migratory wildfowl (in particular geese) make significant contributions to nitrogen and phosphorus loading in wetlands and lakes of a magnitude great enough to cause a upward shift in their trophic status. (Kitchell *et al.,* 1999, Olson 2005, Post 1998). Hence consideration of translocation efforts for *T. intricata,* even if carried out at the isolated points of low nutrient concentration on the site would always be at risk of avian grazing and / or associated nutrient import.

4.1 *Future efforts*

The walking survey of Sculthorpe Moor in June 2008 revealed extensive grassy succession within the ditch where *T. intricata* was first recorded in 2006. This matter should be of some concern, as it was from this ditch that oospore samples were to be taken for translocation to other sites. If this project should move forward in line with BAP targets 2005 (Appendix 2).

a. Efforts should be made to graze or scrape the ditch at Sculthorpe to encourage re-germination of the population.

b. Further work should be carried out to extend the ditches at Sculthorpe moor in the field where *T. intricata* was recorded in 2006. Such work would also be of value in helping to determine the extent and potential of the oospore resource.

c. Sites other than Cantley and Gillingham marshes should be sought for translocation work by a desk study of Norfolk ditch systems and historically recorded water quality, looking specifically to detect spring fed aquifer upwelling points in order to further the project in line with 'option 4' of 'phase 2' of the possible project results scenarios (Fig 1).

5. Conclusion

The results point to rejection of the hypothesis that Cantley and Gillingham Marshes might be suitable candidates for translocation / re-establishment efforts for *Tolypella intricata:* such rejection being based on their February aqueous nitrogen concentrations being above critical limits predicted by recent research.

This brief study has highlighted the value and potential of Sculthorpe Moor as a charophyte site. The results also suggest that once a sensitive species such as *Tolypella intricata* is lost from a site, unless remedial measures are taken to reduce negative influences such as eutrophication and succession, from whatever sources, re-establishment efforts are unlikely to succeed, and hence funding for such projects without rigorous scientific investigation of the site, is unlikely to be well directed. Also re-enforced are the findings of recent research (Lambert 2007) that aqueous nitrogen concentrations are a critical limiting factor for charophyte distribution.

The current lack of attendance to the site at Sculthorpe Moor shows how quickly opportunities may be lost for gaining further knowledge of a rare species habitat, ecology and life cycle. It follows that such sites should be prioritized for investigative and conservation work at both a practical and scientific level.

Tolypella intricata is often described an 'opportunist' colonizer (T. Pankhurst pers com) and its non appearance at sites annually might not be cause for concern. However this viewpoint should be placed in context of the rapid decline in its distribution over the past 50 years within Britain. This general decline must point to the fact that opportunities for germination and persistence are becoming fewer and fewer, as conditions generally become less favorable. As such every effort should be made to conserve the populations and ecological integrity of extant sites such as Sculthorpe Moor.

The valuable resource of a known extensive oospore bank for *Tolypella intricata* at Sculthorpe Moor also provides opportunity for further scientific investigations into the population biology and autecology of the species. This is particularly so this populations lies is within 50 miles of the University of East Anglia, where the ecology of British charophytes has been a key research theme under the guidance of Professor Tony Davy in the school of Biological Sciences since 1998. It is suggested that further funding is sought from lead partners to study the ecology of the species in detail to gain knowledge that might assist attempts to re-establish its national distribution in line with local BAP targets.

In conclusion the evidence of this report suggests that Cantley and Gillingham Marshes are not ideal candidates for re-establishment efforts for *Tolypella intricata*, this being most probably due to the original causes of the loss of the species from them in the first place i.e. eutrophication and guanification: it is recommended that to continue the Norfolk BAP for *Tolypella intricata* (Appendix 2) a desk study is undertaken in 2009 to identify more suitable sites within Norfolk for re-establishment in line with option 4 of phase 2 of this project outline (Fig 1).

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Appendix 1. Survey Points

Fig. 7 Survey points and charophyte recordings at Cantley Marshes (Feb 2008)

Table 4. Macrophyte records (% cover), Nitrate (Nitrogen) and Phosphate (P) at Cantley Marshes in February 2008.

Sample point	Lat	Long	Nat Grid	Species	% Cover	N (mgl^{-1})	P (μgl^{-1})
1	52.58438	-1.49561	TG 36904 04248	None	0	0.25	0
2	52.5853	-1.49807	TG 37065 04358	None	0	1.54	0
3	52.58569	-1.49623	TG 36939 04395	None	0	0.29	0
4	52.58622	-1.48945	TG 36477 04432	None	0	0.52	0
5	52.58715	-1.4916	TG 36617 04543	None	0	1.53	0
6	52.58652	-1.49572	TG 36900 04486	*A. nodiflorum*	10	4.91	0
6	52.58652	-1.49572	TG 36900 04486	*Pot. sp.*	10	1.69	0
7	52.58506	-1.49852	TG 36900 04486	None	0	0.4	0
8	52.58226	-1.5036	TG 37097 04333	*A. nodiflorum*	20	1.69	0
8	52.58226	-1.5036	TG 37097 04333	*M. aquatica*	20	1.69	0
9	52.58235	-1.49687	TG 37000 04026	None	0	2.67	0
10	52.58226	-1.5036	TG 37456 04038	None	0	1.89	0

Fig 8. Survey points and charophyte recordings at Gillingham Marsh Feb (2008)

Table 5. Macrophyte records (% cover), Nitrate (Nitrogen) and Phosphate (P) at Gillingham Marshes in February 2008.

Sample point	Lat	Long	Nat Grid	Species	% Cover	N (mgl^{-1})	P (μgl^{-1})
1	52.46781	-1.55229	TM 41381 91475	*C. vulgaris*	60	0.4	0
1	52.46781	-1.55229	TM 41381 91475	*Spirogyra*	40	0.24	0
2	52.46577	-1.54804	TM 41103 91234	*L. minor*	70	0.54	0
3	52.46417	-1.54845	TM 41140 91058	*L. trisulca*	70	0.88	0
4	52.46613	-1.55468	TM 41152 91296	*None*	0	0.39	0.06
5	52.46761	-1.55307	TM 41435 91456	*C. vulgaris*	80	1.61	0
6	52.46744	-1.55617	TM 41646 91447	*C. hsipida*	80	1.05	0
7	52.46800	-1.55802	TM 41769 91515	*C. vulgaris*	80	0.5	0
8	52.46735	-1.55652	TM 41671 91438	*C. hispida*	80	0.51	0
9	52.46754	-1.558531	TM 41806 91466	*C. vulgaris*	20	0.43	0
10	52.46703	-1.557439	TM 41735 91406	*C. hispida*	60	0.32	0

Fig 9. Survey points and charophyte recordings at Sculthorpe Moor (Feb 2008)
*Point 10 may have been *T. intricata*, formal identification of a new sample is awaited from Nick Stewart in spring of 2009.

Table 3. Macrophyte records (% cover), Nitrate (Nitrogen) and Phosphate (P) at Sculthorpe Moor in February 2008.

Sample point	Lat	Long	Nat Grid	Species	% Cover	N (mgl^{-1})	P (μgl^{-1})
1	52.83748	-0.82532	TF 90401 30403	*T. intricata*	20	0.11	0
2	52.83666	-0.82601	TF 90452 30313	*T. intricata*	20	0.48	0
3	52.83589	-0.82636	TF 90478 30229	*C. vulgaris*	60	0.34	0
4	52.83498	-0.82259	TF 90229 30117	*C. curta*	5	0.47	0.01
5	52.83465	-0.82300	TF 90258 30082	*C. contraria*	5	0.49	0
6	52.83462	-0.82308	TF 90263 30079	*C. virgata*	5	0.16	0
7	52.83335	-0.82260	TF 90236 29936	*C. hispida*	5	0	0
8	52.83332	-0.82160	TF 90169 29930	*C. virgata*	30	0	0.02
9	52.83332	-0.82213	TF 90205 29932	*C. globularis*	5	0.32	0
10	52.83903	-0.81944	TF 89999 30559	*T. glomerata*	80	0.19	0

Appendix 2

BAP Actions (Published 2005)

Species management and protection for *Tolypella intricata*

Undertake experimental management at five suitable historic sites with the aim of regenerating populations from the spore-bank. Management may include scrub clearance and soil disturbance. Suitable historic sites will include those where a long-term management commitment is possible. (ACTION: NE)

Consider undertaking (re)introductions of this species to suitable ponds in historic sites or the vicinity of extant sites, if regeneration from the spore-bank proves unsuccessful. (ACTION: NE).